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NEW PROTIST PARASITES FROM THE INTESTINE OF TRICHOPTERA.

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(With Plate XVIII.)

WHEN examining the contents of the alimentary canal of caddis-fly larvae, I found that in addition to certain well-known gregarines, they generally harbour several other interesting protist parasites which have not hitherto been described. Chief among these are: (*a*) a trichomastix, (*b*) a spirochaete, and (*c*) a flagellate which appears to be related to *Macrostoma caullery*i, Alexieff. The present paper deals with the trichomastix and the spirochaete: I hope to describe the other flagellate fully in a future communication.

(*a*) **Trichomastix trichopterae**, n. sp.

I. *Systematic position.*

The trichomonads form a well-marked group of flagellate parasites, characterized by a skeletal axial rod and four flagella springing from the extreme anterior end of the pear-shaped body; three of these flagella are directed forward, the fourth is either a free, backward-directed "Schleppgeissel" (*Trichomastix*, Bütschli), or is attached along the body by an undulating membrane (*Trichomonas*, Donné).

The genus *Trichomastix* contains three well-authenticated species—*Trichomastix lacertae*, Bütschli, *T. serpentis*, Dobell, and *T. batrachorum*, Dobell. *Trichomastix caviae* (Davaine) is sometimes included here, though Grassi's description of the flagella is rather uncertain. As their specific names imply, these forms are all parasitic in vertebrates. Grassi, however, states that he saw in the intestine of *Blatta* a parasite

similar to *T. caviae*: it is not impossible that some ill-defined species of *Monocercomonas*, Grassi, parasitic in insect larvae, may belong here. At any rate, I am about to describe a typical trichomastix, which occurs regularly and in fairly large numbers in the aquatic larvae of certain trichopterids—the so-called "caddis-worms" of our ponds and streams.

Looking to the habitat of the hosts, I was at first inclined to think that there might be some connection between the trichomastix of frogs and toads, and that of the larval insect. The caddis-worm might easily swallow the cysts of *T. batrachorum* in the course of feeding, and might provide a suitable field for development of that flagellate. The chances, however, are against this—*i.e.* other insect larvae feeding alongside the caddis-worms never showed any infection with trichomonads: then again, *Trichomonas batrachorum*, Perty, which is much the commoner trichomonad in frogs, has no counterpart among the parasites of the insect: lastly, though it is difficult to define the different species of *Trichomastix*, yet I hope to be able to show that the parasite of the trichopterid larva has certain minor features that help to separate it morphologically from hitherto-described species, and justify my calling it *Trichomastix trichopterae*, n. sp.

II. *Material and Methods.*

The larval insects were collected from ponds and streams in the neighbourhood of Aberdeen at various times from April until the end of December, 1909. Specimens from different localities were found to be equally infected.

The forms examined included species of *Limnophilus* (*L. rhombicus*, L., and *L. flavicornis*, L.), *Anabolia*, *Stenophylax*, *Sericostoma*, and *Mystacides*.

The species of *Limnophilus* were always the most richly infected, *Stenophylax* and *Sericostoma* much more slightly, and *Mystacides* never showed any infection. This probably has reference to the feeding-grounds of the hosts, and the resulting degree of suitability of the intestinal contents to the needs of the parasites. For instance, *Limnophilus*, living in stagnant waters, swallows large quantities of bacteria, which multiply extensively in the hind part of the gut, and form excellent pabulum for the flagellates. *Anabolia*, *Stenophylax*, and *Sericostoma* I found mainly in streams, where they feed more "cleanly," and are not nearly so much infected with bacteria. *Mystacides* occurred in slowly running water, where, judging from its intestinal contents, it

makes its diet almost exclusively off algae and diatoms,—bacteria were relatively scarce, and I never saw any trichomonads.

I could detect no differences between the trichomastix from one genus and that from another.

In all, 100 larvae were examined; of these all but 14 were infected in a greater or less degree, and of the latter, 6 belonged to the genus *Mystacides*.

I examined 13 pupae and 20 adult caddis-flies, but never found any trace of the flagellate.

The parasites occupy a very definite position in the gut of the larva. The upper portion of the intestine, from which the Malpighian tubules come off, is considerably wider than those regions of the gut immediately above and below it. There is therefore a tendency for *débris* to collect at this point, and the intestine is sometimes almost blocked by a rich growth of bacteria. Chief among these is a species of *Streptothrix*, its filaments forming dense, tangled masses; a large *Bacillus* also occurs frequently[1]. If the gut be dissected out, and examined under a $\frac{1}{6}$ objective, the flagellates can easily be seen through the semi-transparent walls, moving about among the thick bacterial growth in the upper intestine. They also occur, though in much smaller numbers, in the lower part of the intestine, and in the rectum. I have never seen them in the mid-gut.

In order to study the living parasite, I teased out the intestine in a small drop of normal salt-solution, which I examined under an oil-immersion (Zeiss $\frac{1}{12}''$), after first sealing down the coverslip with wax. The parasites are very active at first after removal from the host, but soon become sluggish in their movements, and die in an hour or less: in this respect they seem more sensitive than *Trichomastix lacertae*, and *T. batrachorum*, which Prowazek and Dobell describe as living under these conditions for a long time. Of the *intra vitam* stains employed, neutral red proved the most useful. Schewiakoff's method of fixation, with osmic acid vapour and subsequent examination in soda solution, was found very useful in determining the number and point of origin of the flagella.

In making permanent preparations, I tried all the ordinary fixatives and stains, but the results given by Heidenhain's iron-haematoxylin after fixation with sublimate-alcohol (Schaudinn's formula) were so much the most satisfactory that I soon trusted to this method alone.

[1] Léger (1902) describes these, or very similar, bacteria from the intestine of the larva of *Chironomus*.

III. *Description.*

(1) *The living parasite.*

Trichomastix trichopterae is of approximately pear shape, with average dimensions of $8{\cdot}5\mu \times 3\mu$. There is great diversity in size, however; some large individuals measure as much as $12{\cdot}8\mu$ in length, and there occur minute forms of only 5μ. From the anterior end arise four long flagella, of which three are directed forwards, and one backwards as a sort of "Schleppgeissel." They are all rather longer than the organism itself, but the "Schleppgeissel" is generally the longest. The point of origin of the flagella is a highly refractive granule situated in the flexible prominence that slightly overhangs the cytostome. The "stalk" of the pear is formed by the axostyle, which projects from the posterior end, and can be traced up for a considerable distance within the body as a refractive rod. The projecting portion is frequently as much as 7μ long, or more than half the total length; it would seem to be longer in proportion here than it is in other species. Sometimes it is much reduced, or may occasionally be altogether absent. It can be seen that the axostyle is clothed by a layer of cytoplasm of somewhat unequal thickness: the appearance reminds one strikingly of the axopodia of the *Heliozoa*. In *Trichomastix trichopterae* the axostyle, while no doubt partly skeletal in function, is also used as an organ of attachment. In this respect it differs from the axostyles in *T. serpentis* and *T. batrachorum*, which, according to Dobell, are purely skeletal[1]. If the intestinal contents of a larva be teased out and examined *in vivo*, many of the trichomonads will be found attached by the tip of the axostyle to clumps and tangles of bacterial matter. Anchored thus, the organism lashes steadily with its flagella, and drives a current bearing food-particles forwards in the direction of the cytostome. This is quite the normal state of affairs, as may be proved by watching the flagellates moving inside the gut. The cytoplasm clothing the axostyle is slightly viscous, especially towards the extremity, to which small masses of bacteria and *débris* can often be seen adhering. At times the organism writhes slowly about among the tangles of *Streptothrix* where the flagella have not free play, and the forward progress seems to be effected by "euglenoid"-like movements of the body. Under unfavourable conditions, such as pressure from the cover-glass, I have seen the

[1] In the *Trichomonas* from the mouse, Wenyon (1907) describes the axostyle as an organ of temporary fixation.

protoplasm of the whole body perform the curious undulations described by authors in various species of *Trichomonas* and *Trichomastix*.

When swimming freely, the flagellate moves with the jerky, hopping action characteristic of trichomonads in general. The three anterior flagella lash backwards and forwards in unison: the swing of the "Schleppgeissel" is not usually simultaneous with that of the rest, but falls a little behind. This flagellum may also describe a sort of spiral, a movement that probably accounts for the frequent rotation of the whole organism.

I was never able to follow the whole process of division on one individual, but I observed a sufficient number in different stages to be certain that the process is similar to that described for other species. The axostyle is withdrawn, and the animal becomes roughly circular in form: then it can be seen that the refractive spot corresponding to the basal granule has become doubled, and that these two then move away from one another towards opposite poles of the now ovoid body. Certain flagella go with each basal granule, but in the living state it is impossible to be quite certain as to their number and arrangement. A clear refractive line can sometimes be seen extending from one granule to the other. The cell now becomes constricted in the middle, and with some rapidity the two halves separate from one another, the connecting portion thinning out more and more until it snaps, and the daughter flagellates separate. The first stages of division are relatively slow, but the final separation is rapid, which probably accounts for the rarity of the final stages in stained preparations. During division the animal's movements are irregular and jerky, and it does not make much forward progress. With regard to encystment I can say very little. Occasionally individuals were seen to attach themselves to clumps of bacterial matter, withdraw their axostyle and apparently round off, but, though watched for hours, they never showed any signs of further development. I never saw anything of the nature of conjugation between free-swimming individuals, though, after some time under the coverslip, the flagellates seem to get sticky and tend to adhere to one another when they touch.

(2) *Stained Material.*

In the stained flagellate the chief difference between this and other species lies in the great length of the axostyle and in the position of the nucleus, which, though sometimes just below the basal granules, is more

often half-way down the axostyle (Figs. 1 and 2). In this way the connection between the basal granules and the axostyle is left very clear. The axostyle generally appears to run through the nucleus, or is very closely applied to it: in a few instances, however (Fig. 3), I found specimens in which the nucleus had been shifted out of its normal median position, and the axostyle lay exposed for its entire length.

I saw no differentiation into forms with thin and forms with thick axostyles, such as Dobell describes for *T. batrachorum*.

Division stages are very difficult to find, and it is only after prolonged searching that I have been able to collect the series shown in Figs. 4 to 10. It will be seen that they agree in all essentials with the figures given by Prowazek and Dobell. I am in agreement with Dobell in thinking that the axostyle is withdrawn and absorbed, and is then reconstructed from the basal granules in the process of division. The early division stages show this quite clearly. The nuclear membrane disappears; the chromatin collects into large, irregular masses; the basal granules separate, and form the poles of the spindle on which the chromatin becomes divided into two portions. At this point the body of the organism shows a median constriction, which deepens until the two halves are hanging together by a thin strand. The final stages of division are rare in my preparations, but Fig. 10 shows the daughter flagellates in the act of separating. It is very difficult to make out the fate of the flagella during this process. Apparently, two go over with each basal granule, where two new are then formed afresh for each individual. Figs. 6, 7 and 8 show the new flagella sprouting. I cannot say for certain what happens to the cytostome. From Fig. 10 it would seem that the old cytostome goes over into one of the daughter flagellates, while the other must form a new one for itself.

The cytoplasm shows a very clear mesh-work structure of varying coarseness. It often contains darkly staining granules and bacterial masses.

I have never seen cysts of the type described by Dobell for *T. batrachorum*. After long search I found in four preparations a few instances of what appear to me to be stages in the encystment of *Trichomastix trichopterae*, though I must admit that, without observation of the living cyst, it is dangerous to be dogmatic. As Dobell (1908) has recently emphasized, there is a risk of confusing with true cysts yeast-cells or other organisms present in the intestinal contents. Figs. 12 to 15 illustrate the objects to which I refer. They bear a striking resemblance, at any rate, to encapsuled organisms of the

trichomonad type. The dimensions are $7{\cdot}5 \times 4{\cdot}5\mu$, and $5{\cdot}5 \times 5\mu$. There is a well-marked cyst-wall. Furthermore, they occur singly, and never in groups such as one would look for in the case of a yeast. In Fig. 12 a single individual appears to be encysted, nucleus, basal granule, and axostyle being all recognizable. Fig. 13 shows two rather large deeply-staining masses of chromatin at opposite poles of the ovoid cyst; between them stretches a dark strand or rod. In Fig. 14 the joining rod is disappearing, and the chromatin masses lie at opposite sides of the cell. In Fig. 15 it can be seen that the cytoplasm shows the beginning of a median constriction. The nuclei are in this case each surmounted by a deeply staining granule. Other granules occur scattered in the dense cytoplasm, but have not the definiteness of these, which I regard as the basal granules. The encysted material was so meagre that I am not able to say what stages these several cysts represent. Possibly the organism multiplies by simple fission within the cyst; or these may be stages in a sexual process, but whether isogamic or autogamic, I cannot say. Prowazek described autogamy in *T. lacertae.* Dobell has thrown some doubt on Prowazek's account, but whether Prowazek were right or wrong in the interpretation of what he saw, the possibility of sexual cysts occurring in *Trichomastix* is by no means excluded by Dobell's description of agamic cysts in *T. batrachorum.*

(*b*) Spirochaete.

In seven larvae[1] a typical slender spirochaete was found in the same region of the gut as the flagellates—*i.e.* just below the Malpighian tubules. The infection was never very rich, but occasionally patches of the large epithelial cells of the gut-wall would appear to be covered with close-set vibratile cilia—the effect produced by a mass of spirochaetes, each attached to the gut-wall by one end, while the free portion vibrated ceaselessly. In teased-out preparations of the gut-contents the spirochaetes were sometimes found singly, but more often in tufts vibrating about a small mass of detritus or bacteria. The usual movements characteristic of spirochaetes in general were observed: *i.e.* rapid screwing round the long axis and flexion of the whole body, especially at a middle point.

The movements are exceedingly rapid and hard to follow at first, becoming slower after a short time and gradually ceasing; in this stage

[1] In one pupa a very few spirochaetes were seen. The adult caddis-flies examined never showed any infection.

the individuals tend to collect in tangled skeins. They never long survived removal from the host, and normal salt solution seemed to hasten death. In dying there is a strong tendency for the curves to become flattened out and more irregular, but I never noticed anything that could be described as plasmotypsis.

On one or two occasions I saw individuals break across, the middle portion gradually becoming drawn out "like a glass rod in a flame," and then snapping, but I am unable to say whether this were a true transverse division or merely the final stage in a longitudinal division.

I fixed smears with absolute alcohol and stained with Giemsa's stain, but had some difficulty in getting good results. This was probably due in part to the obscuring effect of the large amount of bacteria and *débris* in every preparation ; but the spirochaetes themselves did not take on the stain easily. Fig. 16 shows typical slender spirochaetes, drawn out finely at their extremities and stained an almost uniform pinkish-red with Giemsa. The average length is 15μ, with about 3—6 somewhat unequal curves. I saw no hint of an undulating membrane, nor could I make out any nuclear structure, though sometimes there appeared to be an alternation of slightly lighter and darker areas (Fig. 16*a*).

This spirochaete from the larvae of Trichoptera agrees closely with that described by Léger (1902) from larvae of *Chironomus*, and by Léger and Duboscq (1909) from the larva of *Ptychoptera contaminata*. It may very possibly be identical with these, and without further evidence, I prefer not to create a new species for it.

In addition to the spirochaetes in the two larvae just mentioned, the only other insect spirochaetes of which I can find record are *Spirochaeta culicis*, Jaffé (1907), from the larva of *Culex*[1], and *S. glossinae*, Novy and Knapp, from *Glossina palpalis*.

The record of a new insect host for spirochaetes is therefore not uninteresting.

[1] Ed. and Et. Sergent found spirochaetes in the larva of *Anopheles maculipennis* in Algiers ; Patton states that he has found them abundantly in mosquitoes in India ; and it may also be well to mention in this place the much-disputed *Spirochaeta ziemanni*, Schaudinn, from *Culex pipiens*.

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EXPLANATION OF PLATE XVIII.

All figures drawn to scale, using Zeiss' $\frac{1}{12}$" achromatic objective and ocular 4. Fig. 16 is drawn on a slightly smaller scale than the rest.

Figs. 1–15 stained with iron haematoxylin, Fig. 16 with Giemsa's stain.

(*a*) *Trichomastix trichopterae.*

Fig. 1. Typical individual with long axostyle, clearly connected with basal granules.

Fig. 2. Individual showing nucleus placed far down the axostyle.

Fig. 3. Individual with displaced nucleus exposing axostyle from end to end.

Fig. 4. Withdrawal of axostyle and irregular disposition of nuclear chromatin previous to division.

Figs. 5–10. Stages in longitudinal division. Fig. 10 illustrates the final stage, with the daughter individuals hanging end to end.

Fig. 11. Flagellate with short axostyle, and nucleus showing central karyosome.

Figs. 12–15. Cysts of *Trichomastix trichopterae*.

(*b*) *Spirochaete.*

Fig. 16. Groups of five spirochaetes from hind-gut of trichopterid larva. Fig. 16*a* shows an individual with alternating dark and light transverse bands. Fig. 16*c* appears to be a case of transverse, and Fig. 16*d* of longitudinal division.

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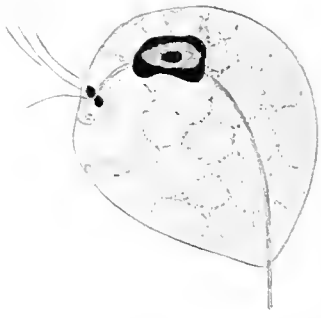

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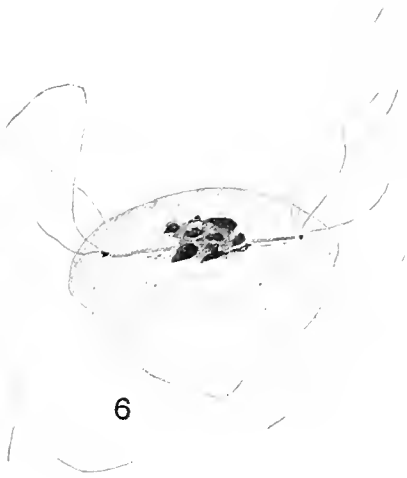

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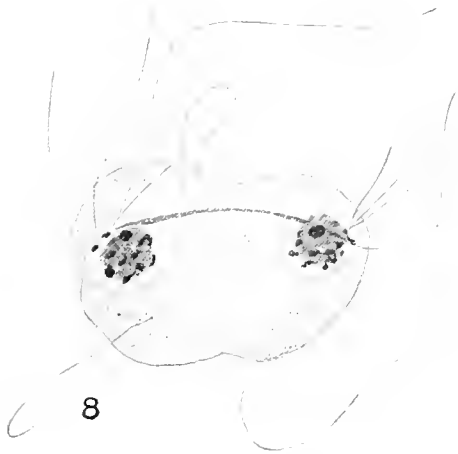

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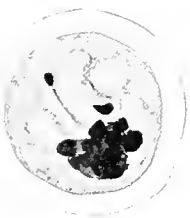

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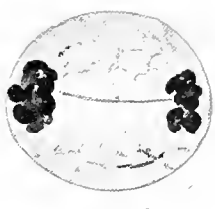

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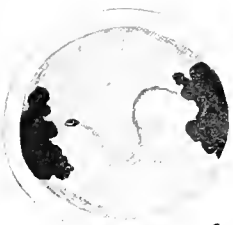

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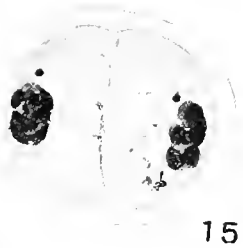

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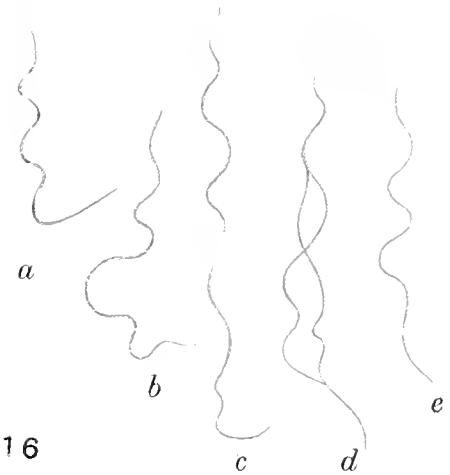

16

HERPETOMONADS FROM THE ALIMENTARY TRACT OF CERTAIN DUNG-FLIES.

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(With Plate XIX and 4 Text-figures.)

Introduction.

In 1903 Léger pointed out that a flagellate apparently identical with *Herpetomonas muscae-domesticae*, Burnett, may occur in other species of flies frequenting the neighbourhood of houses. The species found to be infected were *Homalomyia scalaris*, F., *Pollenia rudis*, F. and *Theicomyza fusca*, Macq. Beyond certain slight variations of form, the parasites from the different hosts showed no noteworthy morphological deviation from the type species.

Roubaud (1908) has also recorded *H. muscae-domesticae* as occurring along with *Herpetomonas* (*Leptomonas*) *mesnili* from species of *Lucilia*, and with *Herpetomonas* (*Leptomonas*) *mirabilis* from *Pycnosoma putorium*.

The list of herpetomonads from insects steadily increases, and there is at present too great a tendency to regard each new herpetomonas "find" as a separate species peculiar to the host. Where the generic name of the host is used in forming the specific name of the parasite this custom is convenient enough, affording as it does a ready index of distribution: where the specific name is more fanciful, there is less to be said for it. In either case, until more of the known forms have had their life-cycles worked out, it must be admitted that the arrangement is artificial and should be looked on as tentative.

I have recently found flagellates resembling *Herpetomonas muscae-domesticae* in three dung-flies—*Scatophaga lutaria*, F., *Neuroctena anilis*,

Fallen, and *Homalomyia*, sp. (probably *H. corvina*, Verrall[1]). The material was sufficiently abundant to admit of my working out the life-cycle, and I found that the herpetomonads of these three flies were at each stage of their development morphologically indistinguishable from one another and from *H. muscae-domesticae*, so far as I have been able to study that species.

I regard *Musca domestica* and other non-biting flies frequenting similar feeding-grounds as subject to infection by a common flagellate. In view of the known polymorphism and great plasticity of herpetomonads, it seems more logical to refrain here from the multiplication of species, and to regard slight deviations from the *muscae-domesticae* type as no more than might be looked for in response to the slightly different environment[2]. One is tempted to doubt whether forms such as *Herpetomonas lesnei*, Léger, *Herpetomonas sarcophagae*, Prowazek, and *Herpetomonas* (*Leptomonas*) *drosophilae*, Chatton and Alilaire should really rank as distinct species[3], and whether *Herpetomonas* (*Leptomonas*) *mesnili*, Roubaud, and *Herpetomonas* (*Leptomonas*) *mirabilis*, Roubaud, are not slight variations of one peculiar form.

In studying the herpetomonads of flies it is necessary to keep in mind that one rarely finds more than one stage well represented in the gut of an individual insect. It follows therefore that without careful study of a sufficiently large number both of the adult flies and of their larvae, the account is too incomplete to be of much value except as a record of distribution.

So far as I am aware, Patton (1909) is the only observer who has succeeded in tracing out the life-cycle of a herpetomonad from a non-biting fly. I am glad to be able to substantiate his account from what I have seen in the three species of dung-flies that I examined. A rich material has enabled me to treat certain points in fuller detail.

Material and Methods.

The flies were caught during the summer months near a pond in the neighbourhood of Aberdeen, where they were usually found feeding on

[1] I am much obliged to Mr Percy Grimshaw for kindly identifying these flies.

[2] Alexieff (1909) makes similar observations on the variability of the flagellate parasites of amphibians.

[3] Chatton and Alilaire would of course object that their flagellate had but one flagellum and is therefore not a *Herpetomonas* in their sense of the name. I shall return to this point presently.

human excrement. The rate of infection was pretty high—about 60 %. The parasites were always confined to the alimentary canal. The larvae of *Scatophaga lutaria* and of *Homalomyia* from the same feeding-grounds were even more richly infected than the adult insects. In the autumn I took a large number of these larvae into the laboratory, and kept them under observation at a temperature of 60°—70° F., with a view to finding out what changes were undergone by the parasite during the insect's development. Most of the larvae pupated, and a number of flies came out after about three weeks.

I made attempts to cultivate the herpetomonads on agar-agar. The flagellates were taken from the gut of both kinds of larvae. They lived for two or three days on the new medium, but without multiplying, and in nearly every case assuming a rounded up or semi-encysted condition: the bacterial growth by that time had become so great that it was found impossible to isolate the flagellates in culture, and the attempt was abandoned.

The gut of the fly or larva was examined for parasites in the usual way—*i.e.* it was dissected out in a drop of normal salt solution and examined under the microscope. If the parasites were present their characteristic movements betrayed them at once, even under a low power. The gut was then divided into three portions, and teased out. For Giemsa preparations I tried fixation with osmic acid vapour and with vapour of 40 % formation, as well as the usual dry method. For adult flagellates I found the dry method exceedingly good, but for encysting and encysted stages the wet method gave much less deformation.

While admitting the great value and brilliance of the Romanowsky stains, I am of opinion that a more reliable cytological stain, such as iron-haematoxylin, should be used as a control wherever possible. Herpetomonads stained with iron-haematoxylin often present a very different appearance from those stained with Romanowsky stains (either after dry or wet fixation)—a circumstance that is at least suggestive in view of the interpretations placed on certain highly dubious structures described by authors from Romanowsky preparations.

For iron-haematoxylin preparations, I spread the gut contents in a thin film on a cover-glass, and dropped it on the hot fixative (Schaudinn's sublimate-alcohol) after the method recommended by Schaudinn. The stain did not always act very satisfactorily, but occasionally, when I got a good differentiation, the result was excellent and was an interesting commentary on the Giemsa preparations.

I place great importance on the study of the living organism. I tried neutral red and methylene blue as *intra vitam* stains, but did not find them helpful: with good lighting, all the essential structures are quite clear on the unstained flagellate.

Life-cycle of Parasite and Mode of Infection of Host.

The parasites were always found in the alimentary canal of the fly. Careful search failed to show any infection of the ova, nor were flagellates ever found in or near the ovaries. This points to the infection being "casual"—*i.e.* by accidental ingestion of the cysts with the food. The high percentage of infection is scarcely surprising, considering the feeding habits. The flies crowd thickly over the surface of the dejecta, abandoning it only when startled, and returning as soon as possible to continue their meal: their food must quickly get thoroughly contaminated with herpetomonad cysts from their droppings. In this connection it is interesting to note that the *Neuroctena*, which is the most heavily infected, is also the most sluggish, and rarely flies to any distance from its feeding-grounds: if not actually feeding, it may usually be found in the close vicinity on the leaves of low-growing shrubs. The flies examined were mostly caught in the months of June, July and September. As they were frequently seen *in coitû* upon their feeding grounds, I thought it not impossible that their larvae would also harbour the parasite. This proved to be the case. In the end of September the patches of excrement on which the flies had been caught were found to be a moving mass of infected dipterous larvae, mainly of *Scatophaga lutaria* and *Homalomyia*, sp. As was to be expected, the infection in the larvae, remaining as they do on their feeding-grounds throughout, was both more frequent and very much more intense than in the adult insects. As Patton has suggested, the degree of infection probably depends directly on the number of cysts ingested. Examination of larvae of all sizes showed me that infection may occur at any period of larval life, even very young larvae being richly infected. It is difficult to find the stages showing formation of the adult flagellate from the ingested cysts. When such stages occur they are found in the upper end of the mid-gut. Enormous multiplication of the flagellate takes place throughout the length of the mid-gut, so that in places the dark-brown gut contents are almost replaced by a seething mass of parasites. When the larva stops feeding previous to pupation, the flagellates begin to round up and collect in the hind-gut, where they encyst. The

majority are passed out with the faeces, but a few half-encysted forms may be found attached to the disintegrating gut-epithelium during the metamorphosis. Of a number of flies I examined on emerging from their pupal cases and before they had fed, only one was found to contain a few half-encysted flagellates in the hind-gut.

I think it likely therefore that the flies reinfect themselves when they begin to feed. *The infection of the adult fly, is probably not to any extent directly continuous with that of the larva, but is freshly acquired.* The cycle in the fly follows much the same course as in the larva. Pre-flagellate stages are infrequent, but when they occur are found in the mid-gut along with the adult flagellates. Rounding up stages occur in the intestine. By far the most common condition was that in which the rectum was full of enormous numbers of encysting and encysted forms. The cysts were found in the faeces. All the stages were never seen at one time in one host.

It will be seen from the above that the mode of infection and the behaviour of the parasite in the adult flies of *Scatophaga lutaria*, *Neuroctena anilis*, and *Homalomyia* agree with Patton's account of *H. muscae-domesticae* in *Musca domestica*. That author, however, while stating that infection is probably not hereditary, gives no information regarding larval infection, and one is left to conclude that only the adult flies harbour the parasite. Prowazek (1904) found *H. muscae-domesticae* in the ovaries of *Musca domestica*, and records larval infection.

I have examined a considerable number of larvae of *Musca domestica* and have never found a trace of infection—a great contrast to the condition of things in *Scatophaga* and *Homalomyia*, where the larvae are seldom quite free from the parasite.

Description of Parasite.

The morphology of *Herpetomonas muscae-domesticae* has received much attention from many skilled observers. The flagellates forming the subject of the present paper agree closely with the type-species, but in looking through a large material, I have noted certain points that seem to me worth recording.

A. *Stained material.*

Patton has divided the life-cycle of herpetomonads and allied forms into three periods—(1) pre-flagellate, (2) flagellate, and (3) post-flagellate. I propose to follow this arrangement.

1. *Pre-flagellate.* This stage was found occasionally in small groups and clusters in the mid-gut of the fly, and more rarely in the upper end of the mid-gut of the larva. Its comparative infrequency suggests that flagellation is a rapid process. I tried to induce freshly hatched-out flies to feed on infected food-material, in the hope of studying more closely the details of flagellation, but this attempt was unsuccessful. Pre-flagellate stages in Giemsa stained preparations are small round or oval bodies—$3\mu \times 2{\cdot}5\mu$ to $4\mu \times 3\mu$ (figs. 1—8)—the cytoplasm staining intensely blue, the circular nucleus a uniform deep red: the kineto-nucleus[1] is a small deeply-staining rod-shaped body, either immediately anterior to the nucleus or slightly to one side of it: numerous smaller chromatin-like granules are scattered through the cytoplasm. The future position of the flagellum is usually already indicated by a rose-pink area extending from the kineto-nucleus to one end of the cell. Occasionally the flagellar root can already be seen forming within this flagellar vacuole as a darker-staining strand. The whole cell is in some cases surrounded by a definite cyst membrane, staining deep pink, but more often this has already dissolved. The flagellum comes to the exterior as a delicate pink "brush," which rapidly takes on the more definite appearance of a stout flagellum, and proceeds to elongate. Division may take place even at this early stage (see fig. 8).

2. *Flagellate.* This was by far the most common stage in the larva, where flagellates were found in enormous numbers in the mid-gut; in the adult fly they were not so abundant, but when present, were always in the mid-gut.

The adult flagellates in the larva differ somewhat from those in the fly (cf. figs. 11 and 26). The flagellate in the fly (fig. 11) differs in no essential from *H. muscae-domesticae,* the appearance of which, in Giemsa preparations, is well-known. The average dimensions of a full-grown flagellate are $25\mu \times 2{\cdot}5\mu$ (not reckoning the flagellum). The body is roughly cigar-shaped, slightly blunt behind, and furnished anteriorly with a long (30μ), relatively thick flagellum. The flagellum arises from the neighbourhood of the kineto-nucleus, but its actual origin seems to be in a minute granule in front of that body: to this granule the term "blepharoplast" might be more consistently applied. The root of the flagellum within the body of the organism is about 4μ long and it is

[1] I propose to follow the nomenclature of Minchin (1908) which has since been adopted by others. The term "blepharoplast," if used at all, ought in this case to be reserved for the granule at the base of the flagellum.

sometimes markedly thickened. In Giemsa preparations there is often a small granule at the point of emergence of the flagellum: this does not appear in staining with iron-haematoxylin, and is perhaps due to a deposit of the Romanowsky stain. The rhizoplast and flagellum are very much thicker in Giemsa than in iron-haematoxylin preparations: with regard to the flagellum, the Giemsa certainly gives the proportions more as they are in life. The kineto-nucleus is a tongue-shaped or rod-shaped body, placed about 6μ from the anterior end. In Giemsa preparations it is very large and conspicuous, measuring as much as $2\mu \times \cdot 8\mu$: it stains a dark rich red, in the midst of which it is usually possible to make out a more deeply-staining central body. With iron-haematoxylin (figs. 24 and 25), the kineto-nucleus appears smaller, stains uniformly and tends to be circular or rod-shaped, rather than tongue-shaped. The tropho-nucleus lies posterior to the kineto-nucleus, about half-way along the body. It is oval or roughly circular, measuring about $3\mu \times 2\mu$. In Giemsa preparations it stains pinkish-red, and almost always appears to consist of a fine reticulum on which small chromatin granules are distributed. Iron-haematoxylin gives a very different picture: here the stain is mainly taken up by the central karyosome, and there is a well-marked nuclear membrane; sometimes there is a faint net-work suggested between the karyosome and the membrane, but of chromatin granules there is no hint. In Giemsa preparations the cytoplasm stains a clear blue, tinged with purplish in places, and fading into pink in the neighbourhood of the flagellum and kineto-nucleus. Occasionally vacuolated areas appear, and not infrequent, especially in the smaller flagellates, is a clear sinuous line visible between the kineto- and tropho-nuclei and descending thence into the posterior part of the cell. This line corresponds in position with the spiral "Doppelfaden" described by Prowazek in *H. muscae-domesticae*, but I never succeeded in staining it, and am quite at a loss as to its true nature. I have never seen any hint of a cytostome in these flagellates, and am not inclined to regard it as an "intestinal canal[1]." After Giemsa's stain the cytoplasm appears full of deeply-staining, chromatin-like granules, especially numerous in the region posterior to the tropho-nucleus. That these are not composed of chromatin, however, is well seen on staining with iron-haematoxylin, which the cytoplasm takes on very uniformly, showing a hint indeed of reticular structure but very rarely containing anything that could be called granules. It is probable that

[1] Cf. Léger (1902), *Compt. rend. Acad. Sc.*, cxxxiv. p. 781.

these bodies are of the nature of reserve food-stuff or of excreta. I was unable to detect the posterior diplosome of Prowazek.

The above is a description of the adult flagellate from the fly: it is well to compare with it the corresponding stage from the larva (fig. 26). Here individuals are smaller, the rhizoplast is much shorter, the kineto-nucleus is smaller, ·8μ, stains more evenly, and tends to be circular, the tropho-nucleus is placed further forward in the body, the granules in the cytoplasm are few in number, and restricted to the extreme posterior end.

The interesting experiments of Miss Porter (1909) leave no doubt that these granules in the cytoplasm of herpetomonads are an expression of the degree of metabolic activity, and must not be taken as an indication of sex. This author states that *H. jaculum* in a highly nutritive medium developed large numbers of refractive granules, and when deprived of food, proportionately few. In the case of the flagellates at present under discussion, the smaller size and clearer cytoplasm of the parasites from the larval gut may well be explained by their much denser crowding there and the relatively small food supply per individual.

Division is longitudinal. The rhizoplast usually divides first, and it can be seen that each half carries with it a basal granule (figs. 12 and 23). I have never found stages in which the new rhizoplast was growing up from the basal granule. I am therefore inclined to believe that to this extent there is splitting of the flagellum root. Beyond this point, however, I consider that the second flagellum is a new formation, and does not arise by splitting of the original flagellum[1]. Figs. 12 to 17 illustrate this very well. For some time the two flagella lie closely together, and this may last until the new flagellum has almost reached the length of the first, producing the deceptive appearance of a bi-flagellate organism. This is most frequently the case in the parasite in the fly. In the larva, for some obscure reason, the splitting of the flagellate body usually takes place at an earlier stage in the formation

[1] There is great diversity of opinion as to the mode of origin of the new flagellum in *H. muscae-domesticae* and allied forms. Prowazek (1904) describes the new flagellum in *H. muscae-domesticae* as growing up from the divided basal granule, which he figures at the point of emergence of the flagellum: from this later on in like manner a new rhizoplast grows down. Patton (1909), referring to the same flagellate, finds that longitudinal fission begins with a splitting of the flagellum root. Miss Porter (1909) states that as in *H. jaculum* she has "watched the flagellum of the living *H. muscae-domesticae* divide in two." My own observations on this parasite are in agreement with those of Berliner (1909) on *H. jaculum*.

of the second flagellum. Thus there are comparatively few bi-flagellates seen (fig. 28); much the most common appearance is that in figs. 18 and 19, where the daughter organisms are hanging end to end, the one with a long flagellum, and the other with no more than a short stump yet formed. During the splitting of the rhizoplast, the kineto-nucleus can be seen to undergo division. In the iron-haematoxylin preparations it frequently appears kidney-shaped or tri-lobed at this stage, but an attenuated dumb-bell shape is also seen. Within the kineto-nucleus in Giemsa preparations the central body can be seen dividing in dumb-bell fashion. I have never been able to make out any karyokinetic figures in the tropho-nucleus as stained with Giemsa: it stains a uniform dull red at this stage, or shows the same appearance as in the resting stage. With iron-haematoxylin, however, it can be seen that at a very early stage the karyosome becomes elongated in the direction of the long axis of the flagellate, and the nuclear boundaries become vague. Gradually the ends of the now dumb-bell shaped karyosome recede further and further from one another, until they are only connected by a very faintly-staining strand, which then breaks (figs. 21—24). I never saw any unequal division, but as the exceedingly rapid multiplication proceeds, it is not surprising that the daughter flagellates tend to get smaller and smaller in size. At a certain stage, possibly determined by "depression" following on long-continued multiplication, the organisms proceed to encyst.

3. *Post-flagellate.* The attachment of the herpetomonad flagellates to the epithelium of the hind-gut, their rounding up to form "gregarine" stages and finally cysts, are processes that have often been described. I have little new to add, though it was very common to find the rectum of *Neuroctena* simply swarming with encysting herpetomonads. I would point out, however, that here, as elsewhere, iron-haematoxylin gives a very different picture from Giemsa (cf. figs. 34 and 37 with figs. 39 and 40). The kineto-nucleus appears much smaller, and the nucleus, instead of staining diffusely or appearing to have all the chromatin concentrated around its margin, shows a distinct central karyosome surrounded by a clear space bounded by a nuclear membrane. The numerous "chromatoid" granules in the cytoplasm of Giemsa preparations are here practically absent. A word about the disappearance of the flagellum. In some species of *Herpetomonas* the flagellum is apparently "cast off" in the last stage of encystment: in others, the portion external to the cell fades away and is absorbed. In his account of *H. muscae-domesticae*, Prowazek (1904) speaks of the flagellum as

being withdrawn into the cell. Patton (1909), also referring to *H. muscae-domesticae*, says the flagellum degenerates and is shed. Rosenbusch (1910) describes the withdrawal of the flagellum into the cysts of *Crithidia muscae-domesticae*, Werner. In the flagellates from *Neuroctena* and *Homalomyia* the kineto-nucleus travels back, taking the end of the flagellum with it, till it comes to lie alongside or even behind the tropho-nucleus. In this way the greater part of the flagellum is actually drawn into the interior of the cell, where it is afterwards absorbed (see figs. 33 to 37). The position of the kineto-nucleus at these stages produces a crithidia- or even a trypanosome-like appearance, but there is, of course, no hint of an undulating membrane[1]. Frequently the connection between the kineto-nucleus and the end of the flagellum is lost, and the kineto-nucleus wanders into a different part of the cell.

On one occasion only did I see a glutinous cyst-wall formed, such as has been described by Prowazek and others (fig. 38). It appeared as a faint pink cloud round a large circular cyst, in the centre of which was the tropho-nucleus, surrounded by a number of small, deeply-staining granules.

The final stages of encystment were not often met with. They are small, oval or circular bodies ($3\mu \times 3{\cdot}5\mu$—$4\mu \times 2{\cdot}5\mu$), the cytoplasm staining a deep blue with Giemsa, and containing a dark-red tropho-nucleus and a kineto-nucleus: a reddish cyst membrane could sometimes be made out. Fig. 41*b* shows such a cyst stained with iron-haematoxylin.

Conjugation.

Curious flagellates were occasionally found in Giemsa preparations both from the larva and from the fly, where the tropho-nucleus had disappeared, leaving the cytoplasm staining rose-pink (figs. 29—31). Otherwise these forms seemed to be quite normal, though some of them were very small (fig. 30). I was interested to notice in one case (fig. 29) that division could apparently take place in the virtual absence of the tropho-nucleus. The kineto-nucleus was dividing, as was also the base of the flagellum; two minute, faintly-staining dots alone indicating the normal position of the divided tropho-nucleus.

Such individuals would be regarded by certain authors as male gametes. I have watched the living organisms for hours at a time, and have never seen any sign of conjugation. For my part, I consider these

[1] One cannot help being struck by the resemblance of some of these stages to the flagellate of *Crithidia muscae-domesticae* as figured by Werner.

forms as degenerate, and without any sex significance. I see that Berliner (1909) also interprets as degenerate similar flagellate individuals of *Herpetomonas jaculum*, Léger. I must admit, however, that on two occasions I met with appearances in my Giemsa-stained material that might be looked on as the beginning of conjugation of male and female individuals. Fig. 20 shows such a case. The large half-encysted individual, with pale-blue vacuolated protoplasm, the kineto-nucleus indistinguishable from other darkly-staining granules in the cell, and with the tropho-nucleus situated to one side and apparently extruding chromatin, might be the female, while the small flagellate form alongside, without tropho-nucleus, and with its cytoplasm staining pale-pink, might be the male gamete. On the other hand, the juxtaposition of these apparently sexual individuals might be mere chance. If conjugation be a regular occurrence in this group of flagellates, it is at least surprising that we have hitherto had so little convincing proof of the act itself. Authors who arbitrarily fix on "male" and "female" characters do not give sufficient consideration to the effects known to be produced in protozoa by periods of long continued multiplication[1]. Nuclear hypertrophy, nuclear absorption, the production of undersized individuals, and the suppression of cell-division, resulting in the formation of abnormally large individuals, are all well-known signs of degeneration in protozoa, and have to be reckoned with. It is true, of course, that conjugation may be resorted to as a means of restoring the karyoplasmic equilibrium, but I hold that in the case of the herpetomonads and their allies, we have insufficient proof of this. It should be remembered that encystment is another process that may be made use of by protozoa for self-regulation after periods of prolonged multiplication and consequent depression. This regulation may be effected by extrusion of chromatin

[1] In Hindle's recent very interesting work (1909) on *Trypanosoma dimorphon*, he tentatively distinguishes, on morphological grounds, male, female, and indifferent forms. It is interesting to notice that the "female" is formed from the "indifferent" trypanosome by a process strongly suggestive of the beginning of cell degeneration. The sluggish movements, "stumpy" form, very dense protoplasm containing chromatoid granules, the very large, densely-staining tropho-nucleus, and the frequent extrusion of chromatin from the nucleus, are all features that one is accustomed to associate with cell degeneration. Further, these forms do not occur during the earlier stages of the attack; that is to say, they appear only after the organism has been multiplying by division for some considerable time, and when we might reasonably expect to find some sign of "depression." Hindle himself states that these "female" forms may be found "in all stages of degeneration" in the blood of the rat, but he evidently considers that this would not occur if the conditions were favourable for conjugation: such conditions he thinks might be offered by an intermediate host.

within the cyst, or by the more elaborate processes of autogamy with their attendant karyoplasmic readjustments. The figures and descriptions given by authors (Patton, Berliner, 1909), though not conclusive, suggest that any sexual process may be looked for in the cyst, and is possibly an autogamy.

B. *Observations on the living organism.*

The study of stained material alone is often misleading, and should always be supplemented, where possible, by observation of the living organism. I cannot help thinking that, if this method had been resorted to in the first instance, *Herpetomonas* would now have been described as having a double flagellum[1].

In the case of the fly, one is often able to keep the flagellates under observation in their natural medium, for the wall of the gut is sufficiently transparent to admit of accurate observation of its contents. In the larva this is usually not possible, as there is a great abundance of dark-coloured semi-fluid material in the gut. It is very interesting to watch the flagellates attach themselves to the wall of the intestine, and collect there in groups and clusters during the early stages of the encysting process.

More often I simply teased out the gut-contents in a drop of normal salt solution, and sealed the coverslip well down with wax. The flagellates live under these conditions for as long as 36—40 hours. The adult flagellate moves with extreme rapidity, the body rigid and vibrating like a compass needle from a point about half-way down its length, under the ceaseless lashing of the stout flagellum[2]. Very conspicuous are a number of strongly refractive granules in the posterior end of the body. With good lighting the tropho- and kineto-nuclei and the rhizoplast can also be clearly seen. I have frequently watched the process of longitudinal division. The second flagellum can be seen as a short process springing up at the base of the old flagellum: it increases rapidly in length, and follows the movements of the old flagellum exactly, as though the two were enclosed within the same sheath, or else simply adherent to one another. In this condition they may remain for some time. The body of the animal now begins to split longitudinally, starting from the anterior end, and the two flagella

[1] Berliner (1909) and Porter (1907) have given accounts of the division of *Herpetomonas jaculum in vivo*.

[2] The movements of *Herpetomonas* have recently been well described by Porter (1909).

are torn apart: by now they are usually so nearly equal in length that their independent movements tend to separate the daughter flagellates quickly from one another. This is the condition of things in the parasite from the fly. In that from the larva, as I have said, longitudinal splitting of the body often takes place before the new flagellum has grown up very far (text-fig. 1). The consequence is that the two daughter flagellates tend to hang together by their ends until such time as the new flagellum has grown sufficiently long and strong to work against the old one and so effect the separation. It was only by studying these processes in the living organisms that I came to understand the apparent rarity of the final stages of division in the fly, and their great abundance in the larva.

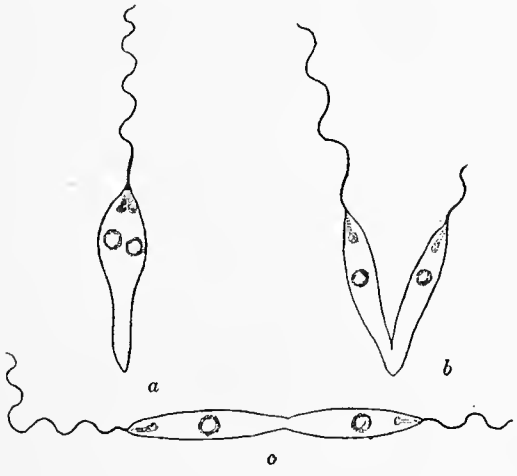

Fig. 1.

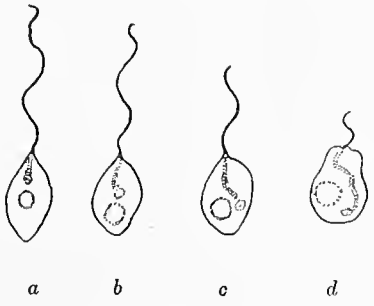

Fig. 2.

After two or three hours the movements of the flagellates have become very much slower, and it can be seen that the body is shorter and more rounded, and has lost much of its rigidity. The flagellum has also become shorter, partly through actual loss of length, but chiefly through being drawn into the body, where it can be traced as a refractive line running back to the kineto-nucleus, now situated near the anterior border of the tropho-nucleus (text-fig. 2). This withdrawal of the flagellum continues until a mere stump is left protruding, and the kineto-nucleus is generally lost to sight among the refractive granules in the hinder part of the now pear-shaped cell. All forward progression of the organism gradually ceases, but the flagellar stump continues to move jerkily to and fro for hours. I have seen division take place at this stage, the longitudinal split starting posteriorly and running

forward, and the flagellum going over to one of the daughter halves without a second flagellum being formed: the halves hung together for a long time and I did not see them finally separate. The organisms all died at this stage, and I never saw the encystment completed.

While the flagellum is being withdrawn, and the body is assuming its pear-shaped form, the cytoplasm becomes rather sticky, and I noticed a strong tendency for flagellates to adhere to one another side by side, either in groups or in pairs. This was often suggestive of the preliminaries for conjugation, but though watched for a long time, these chance groupings never went any further: the organisms either separated after a brief struggle, or else died. It is not infrequent for individuals in this condition to become bent on themselves, the adjacent portions of the body-wall then adhering to one another (see text-fig. 3, *a* and *b*) and producing an appearance not unlike the first stages in the rounding off

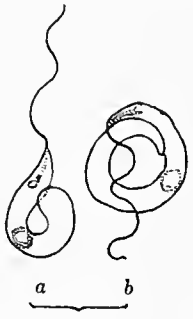

Fig. 3.

Fig. 4.

of *Trypanosoma vittatae* as observed by Robertson (1909). I never found, however, that they developed further. Another instance of the "stickiness" of the cytoplasm was sometimes shown, where the flagellum had got bent back along the body, and adhered firmly to it. Where the flagellum was still pretty long and active, its efforts to continue movement under these abnormal conditions resulted in the gradual lifting up from the cytoplasm of the body of a sort of *pseudo* undulating membrane (text-fig. 4, *a* and *b*). The movements of this structure were so exactly those of a true undulating membrane that it was difficult to believe one was not dealing with a small, blunt trypanosome: observation of the earlier stages of course explained the true nature of the case.

I starved several young larvae of *Homalomyia*, and on examining them found that the parasites had all collected in the hind-gut and were in process of rounding up.

Some of the flagellates that I tried to cultivate on agar-agar continued to live for three or four days, but showed no signs of multiplication. They all assumed the form of the flagellates under the sealed-down cover-slips—*i.e.* the body had become pear-shaped and the kineto-nucleus had travelled back into the body, drawing the flagellum with it.

Herpetomonas or Leptomonas?

I believe that the above account throws light on the value of certain so-called generic characters brought forward in the recent discussion on the classification of the herpetomonads and their allies.

It is necessary to recapitulate briefly. From his observations on the flagellate of *Musca domestica*, Prowazek was led (1904) to state that the genus *Herpetomonas*, Kent, possessed a double flagellum.

Patton (1909) showed clearly that this view was erroneous, the two flagella occurring only in individuals in course of longitudinal division. In support of this he mentioned that if a sufficient number of flies be examined, it will be found that, while in some of them almost all the flagellates have a double flagellum, in others the majority have a single flagellum. Further, in tracing out the life-history he showed that the pre- and post-flagellate stages have only one flagellum. Patton has described many other herpetomonads from various insect hosts, and these he finds fall in line with the flagellate of the fly. He therefore includes them all under *Herpetomonas*, that is, a trypanosomatid having a single flagellum, and with the kineto-nucleus placed some distance in front of the tropho-nucleus in the adult form. Donovan (1909) and Porter (1909) confirm Patton's statements.

Prowazek (1909) repeats his statements, and still holds to the view that *Herpetomonas, sensu stricto*, is a bi-flagellate. Still more confusion has recently been brought in by the repeated attempts of certain of the French school—notably Chatton, Alilaire, and Roubaud—to support Prowazek's view by splitting up the genus *Herpetomonas* into two. They would revive *Leptomonas*, Kent for such forms as have one flagellum and no rhizoplast, reserving *Herpetomonas* for those with a double flagellum and a rhizoplast.

These authors are very doubtful about the value of Patton's definitions of the genera *Crithidia* and *Herpetomonas*, contending that the presence of an undulating membrane and the relative positions of the kineto- and tropho-nuclei are features too variable to be depended on. Chatton and Alilaire (1908—1909) prefer to base their classification

on other characters "d'acquisition ancienne et paraissant actuellement soustraits a l'influence du milieu." As such they select the number of the flagella, and the presence or absence of a rhizoplast[1].

My observations on the herpetomonads in dung-flies are in accordance with those of Patton. The formation of the second flagellum is the immediate forerunner of longitudinal division: in the flagellate as it appears in the larva this is still more evident. The bi-flagellate appearance simply depends on the time at which the longitudinal split occurs,—in the parasite in the larva it is, as I have said, usual to find splitting at a very early stage, and bi-flagellates are therefore rare. The cause of this different behaviour in the fly and in the larva is obscure, but I regard it as an expression of the effect of a slightly different medium on the same organism.

Then as to the second "constant" *Herpetomonas* character—the presence of a long rhizoplast: I have showed that the flagellates in the fly are usually provided with a long, well-marked rhizoplast (the thickness, however, depending a good deal on the stain employed), while in the flagellates from the larva the root of the flagellum is very much shorter, and is often no thicker than the flagellum itself[2]. In certain Giemsa preparations the flagellar root does not stain definitely, but appears as a diffuse pinkish area: such cases seem comparable to Chatton's description and figures of *Leptomonas agilis*, but may merely be due to faulty staining (fig. 28).

If the classification of Chatton and Alilaire be accepted, then the only logical conclusion is that I am here dealing with two different parasites—a *Herpetomonas* from the fly, and a *Leptomonas* from the larva. I prefer to regard the parasite as the same throughout, and I consider that the mere fact that the rhizoplast may assume such different appearances under different conditions, does away with its value in classification.

[1] The authors do not explain how those characters are useful with regard to *Crithidia*, which is the genus particularly offensive to their logical sense.

[2] For my part, I am inclined to regard thickening of the flagellum root, *i.e.* "a well-marked rhizoplast," as the beginning of another division. Supposing that the splitting of the flagellum root is the first step in longitudinal fission, it may often happen that a second division is preparing before the first is completed.

The inconstancy of the rhizoplast is also indicated by the fact that two such careful observers as Berliner and Porter working independently on *H. jaculum* in 1909 are entirely disagreed as to whether a rhizoplast is present in this form or not.

Summary.

1. *Musca domestica* and other non-biting flies frequenting similar feeding-grounds, are probably all liable to infection with a common flagellate. The great variability of this form is shown on comparing the flagellates in the larvae with those in the mature flies of *Homalomyia*, sp. and of *Scatophaga lutaria*.

2. Infection is casual—*i.e.* by the mouth. In the case of the dung-flies examined, the larvae ingest faecal matter infected with herpetomonad cysts: the cysts develop into flagellates in the mid-gut, where they multiply with great rapidity: towards the close of larval life, when the larva stops feeding, they round up in the hind-gut, and are for the most part passed out as cysts. A few survive the pupal stage in a half-encysted condition, but it is probable that the infection of the adult fly is usually freshly acquired. The cycle in the fly is similar to that in the larva, and is in agreement with Patton's account of *Herpetomonas muscae-domesticae*. The parasite was never found in the ovaries or ova. Patton's suggestion that the degree of infection depends directly on the number of cysts ingested, is borne out by the much higher rate of infection in the larvae than in the flies: this is not surprising when we remember the complete restriction of the feeding larva to the infected area.

3. It is important to use some reliable cytological stain such as iron-haematoxylin as a control to Romanowsky stains where possible, seeing that very different results are sometimes given by the two methods.

4. The apparent double flagellum is produced in the course of longitudinal division. The new flagellum grows up alongside the old, and is not merely split off from it: this is best seen in the flagellate from the larva, where the body of the organism usually divides at an earlier period than in the fly. Study of the living flagellate is necessary to a clear understanding of the process of division.

5. I have seen no conjugation in the living herpetomonads. Occasionally flagellates were met with in Giemsa preparations devoid of a tropho-nucleus. Such individuals might be regarded as male gametes; it is more likely that they are simply degenerate forms. Sufficient consideration is not given to the possibility of degeneration in richly-nourished, rapidly multiplying protozoa, such as the trypanosomes and their allies.

6. In encystment the flagellum is not cast off bodily, but is drawn down into the cell by the kineto-nucleus, which moves to a position either alongside of, or posterior to the tropho-nucleus. In this way apparent *Crithidia* or even trypanosome forms are produced, but there is no hint of an undulating membrane. Small blunt "trypanosomes" were also produced occasionally by adhesion of the flagellum to the body-wall in the "stickiness" resulting from confinement under a cover-glass: the efforts of the flagellum to free itself raised up an undulating membrane, and produced a very deceptive appearance.

7. The early stages of encystment could be induced by keeping the flagellates under waxed-down cover-slips, where they would continue to live for 30—40 hours. Similar results were got by transferring flagellates to agar-agar, or by subjecting the larval host to starvation for a day or two.

8. From what I have seen, I do not agree with Chatton and Alilaire's suggestion to divide the genus *Herpetomonas* into *Leptomonas* and *Herpetomonas* proper. I do not think that these authors are justified in regarding the double flagellum and the presence of a long rhizoplast as generic characters.

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EXPLANATION OF PLATE XIX.

All the figures were outlined with Zeiss' drawing-apparatus (Abbé), using Zeiss $\frac{1}{12}$" achromatic objective and ocular 4.

Figs. 1—20 and 26—38 are stained with Giemsa's stain, figs. 21—25 and 39—41 with iron-haematoxylin.

Figs. 1—3. Pre-flagellate stages of herpetomonad from dung-flies. Fig. 1 is from mid-gut of larva of *Homalomyia*, sp.?, figs. 2 and 3 from mid-gut of fly of *Homalomyia* and of *Neuroctena anilis* respectively. Giemsa.

Fig. 4 (*a* and *b*). Pre-flagellates from mid-gut of flies of *Scatophaga lutaria* and *Neuroctena*, showing flagellum forming. (*a*) is surrounded by a cyst-wall: in (*b*) this has disappeared. Giemsa.

Figs. 5—8. Further stages in formation of the flagellum. Giemsa.

Fig. 9. Small flagellate. Giemsa.

Fig. 10. Flagellate in which the body has not yet assumed the characteristic form. Giemsa.

Fig. 11. Adult flagellate from mid-gut of fly (*Homalomyia*) showing single flagellum and well-marked "rhizoplast." Giemsa.

Figs. 12—16. Stages in longitudinal division of flagellates from flies of *Homalomyia* and *Neuroctena*, showing splitting of rhizoplast, upgrowth of second flagellum, and division of nuclei. Giemsa.

Figs. 17—19. Further division stages from mid-gut of larva (*Homalomyia*) to illustrate precocious splitting of the body, with the resulting end-to-end arrangement of two individuals, one with a short and one with a long flagellum. Giemsa.

Note:—Owing to the colours employed in the original drawing, the nuclei in Fig. 17 appear light instead of dark.

Fig. 20. Sexual individuals in conjugation? From rectum of larva (*Scatophaga*). Giemsa.

Fig. 21. Flagellate from mid-gut of larva (*Homalomyia*) showing tropho-nucleus in process of division. Iron-haematoxylin.

Figs. 22—24. Further stages in division of flagellates from fly (*Neuroctena*). Note relatively small kineto-nucleus, well-marked tropho-nuclear karyosome, and absence of "chromatoid" granules in cytoplasm. Iron-haematoxylin. Cf. figs. 12—16.

Fig. 25. Adult flagellate from mid-gut of fly (*Neuroctena*). Iron-haematoxylin. Cf. fig. 11.

Fig. 26. Adult flagellate from mid-gut of larva (*Homalomyia*) showing small kineto-nucleus, short rhizoplast, and scarcity of "chromatoid" granules in cytoplasm. Giemsa. Cf. fig. 11.

Figs. 27 and 28. Early division stages in flagellates from larva (*Scatophaga*). In fig. 28 the rhizoplast is practically absent, the second flagellum has grown up almost to full length, and the tropho-nucleus shows a karyosome. Giemsa.

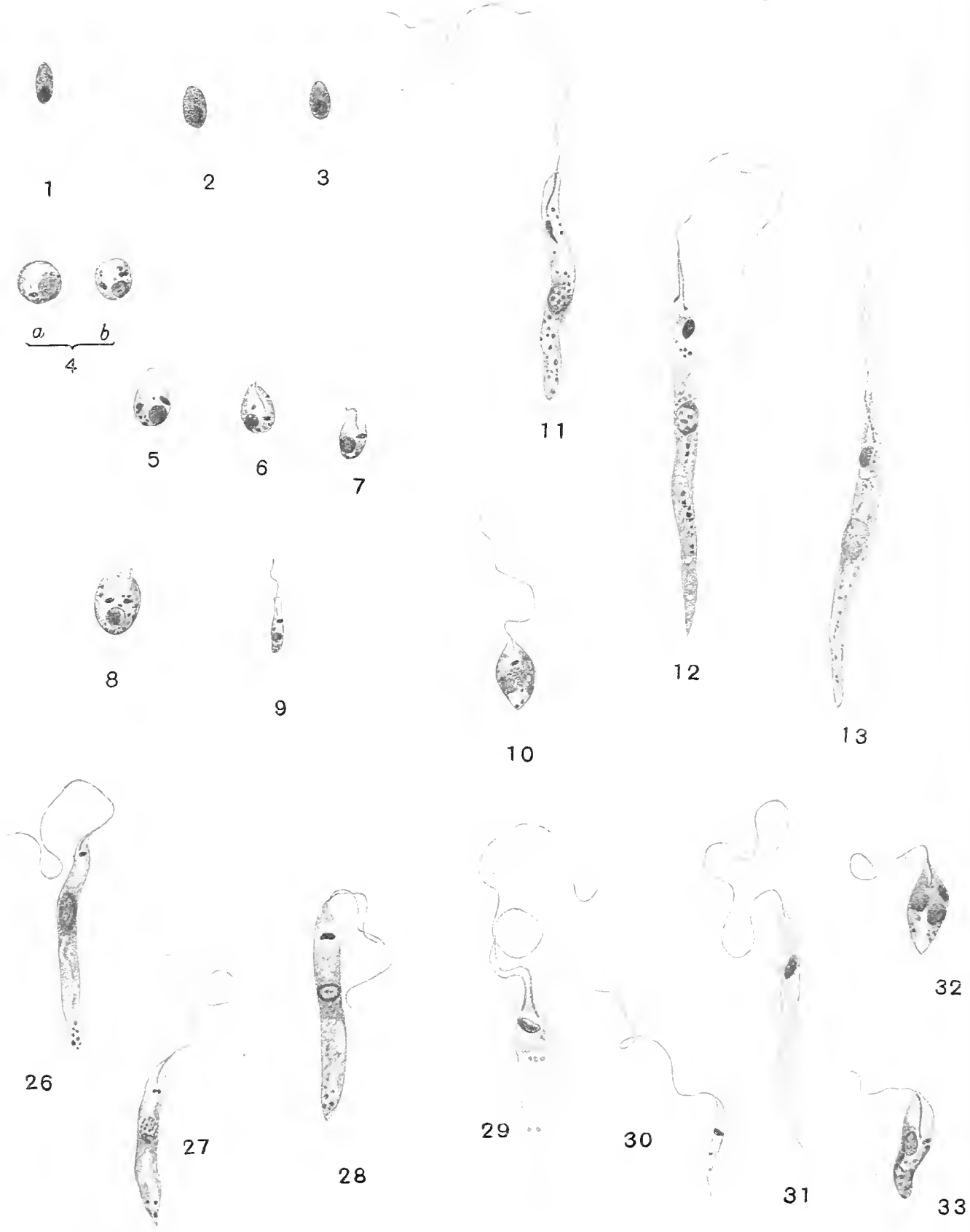
Figs. 29—31. Flagellate individuals without tropho-nucleus. Fig. 29 from mid-gut of fly (*Neuroctena*) and figs. 30 and 31 from hind-gut of larva (*Homalomyia*). In fig. 29 longitudinal division is taking place. Giemsa.

Figs. 32—37. Stages in process of rounding up and encystment in hind-gut of fly. Figs. 34—37 show gradual withdrawal of flagellum and migration of kineto-nucleus into posterior portion of cell. Giemsa.

Fig. 38. Large vacuolated cyst with thick, irregular cyst-wall. Giemsa.

Figs. 39 and 40. Encysting stages. Iron-haematoxylin. Cf. figs. 34—37.

Fig. 41. Post-flagellates from hind-gut of fly (*Homalomyia*). Iron-haematoxylin.



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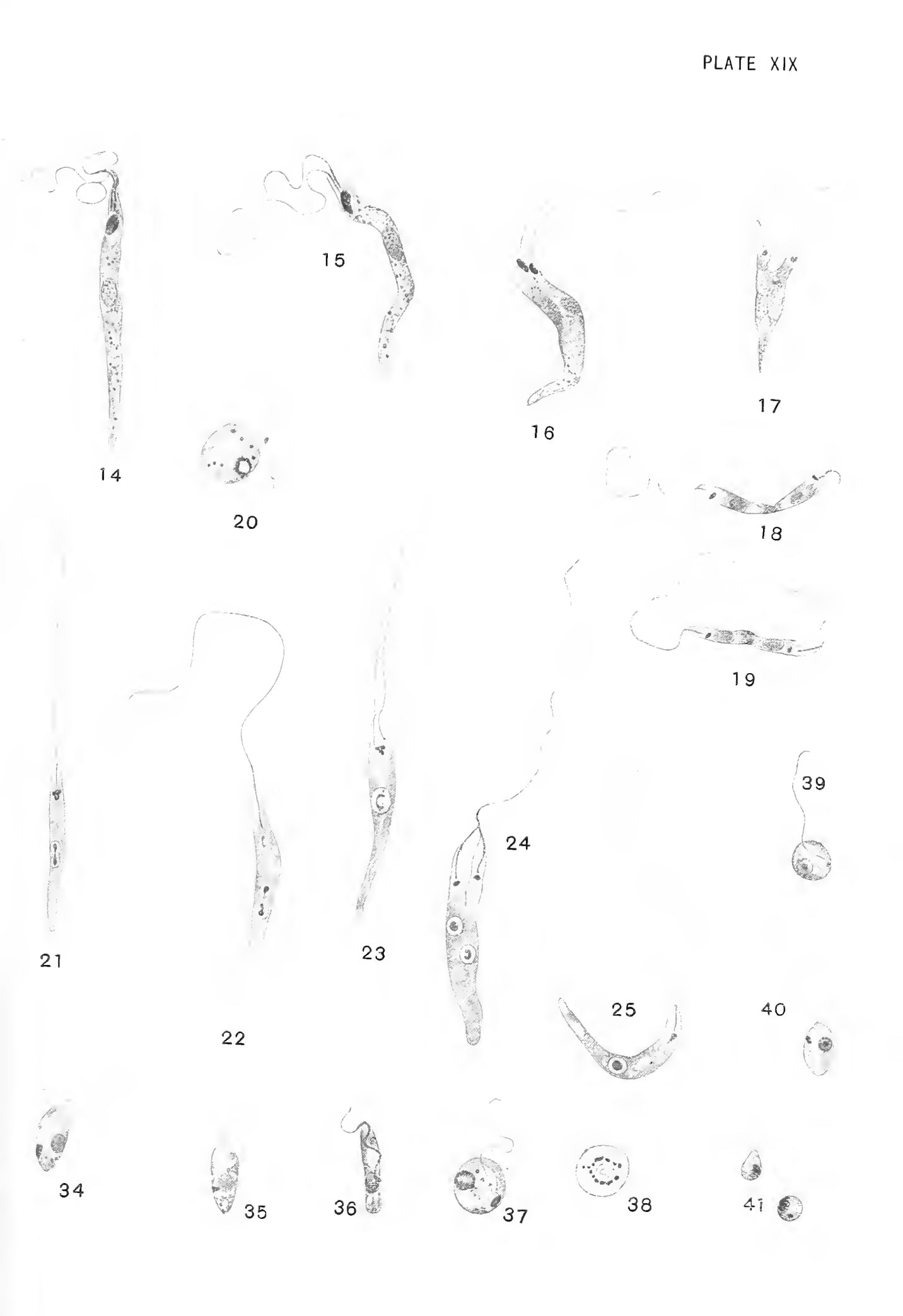
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REPORT UPON TWO SMALL COLLECTIONS OF PENTASTOMIDS WITH THE DESCRIPTION OF A NEW SPECIES OF *POROCEPHALUS*.

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Fellow and Tutor of Christ's College, Cambridge, and Reader in Zoology in the University.

(With Plate XX.)

Through the kindness of Mr Nelson Annandale, Director of the Indian Museum, I have recently had the opportunity of examining a small collection of Pentastomids from that Museum. The collection contains one new species of *Porocephalus* and affords examples of new hosts and new localities in which species already known have been found. In all there were four different species.

(i) *LINGUATULA SUBTRIQUETRA*, Diesing.

This species lives in the lungs of crocodiles and at times wanders into the trachea and pharynx. Diesing described it from *Caiman sclerops* (Hoffmann calls it *Alligator sclerops*), the alligator which has the widest range of all the genus in America. It extends from Southern Mexico to Northern Argentina. As far as I am aware, *L. subtriquetra* has not hitherto been recorded from the Eastern Hemisphere, but the specimen in the Indian Museum was taken from the "pharynx of a crocodile" captured at Saugor on the mouth of the river Hooghly. This species has never been adequately illustrated so I have added some figures which, owing to the skill of Mr E. Wilson, justly represent the characteristic appearance of this Pentastomid. These figures (Pl. XX, figs. 1, 2 and 3) show that there are four papillae in front of the mouth and two behind it, and emphasize the importance of the lateral flaps or flanges.

(ii) *POROCEPHALUS MONILIFORMIS*, Diesing.

Another of Diesing's species described by him from the lungs of *Asterophis tigris* (*Python molurus* and *Python reticulatus*). I have described a specimen from the last-named species which was sent me from Tring. The Indian Museum specimen is simply registered as "from the mouth of a Python."

Owing to the kindness of my friend Mr M. D. Hill of Eton College I have recently had the opportunity of examining a larval *Porocephalus* encysted in the walls of the intestine of *Nycticebus tardigradus*, a new host as far as I know for Pentastomids. This lemur has but the one species and this is confined to the Oriental region. It is not always easy to identify the species of these coiled up, encysted parasites but from the number of rings and the general appearance I think the specimen also belongs to the species *P. moniliformis*.

(iii) *POROCEPHALUS*, sp.?

This species from an unknown host was so shrunken that it was unrecognizable.

(iv) *POROCEPHALUS KACHUGENSIS*, n.sp.

This is I believe a new species and so characteristic that although an encysted and larval form I think it may well be described.

The animals vary a good deal in size, the larger specimens being 12 mm. in length, the small 9 mm. The shape of the body is markedly club-shaped. The thicker or anterior end of the larger animals is 3—3·5 mm. from back to front and 3·5—4 mm. from side to side. The body tapers somewhat suddenly and the posterior two-thirds is from 1—1·7 mm. in breadth and a little less in depth. In their cysts the animals are all coiled up, like a note of interrogation and the coil is always in one plane. (Pl. XX, fig. 5.)

Anteriorly the head bears a pair of well-marked rounded papillae which project forward. (Pl. XX, figs. 6 and 7.) These recall the somewhat similar papillae in *P. megastomus*, Diesing, but in this last-named species there are two others behind the mouth.

The mouth is small and is placed about the level of the base of the inner hooks which are slightly in front of those of the outer hooks. All four hooks project rather further than is the case with most Pentastomids and they are, as Pl. XX, fig. 8 shows, very markedly double. This is also the case with *P. najae sputatricis*, *P. heterodontis*, *P. gracilis* and other species of *Porocephalus*.

The number of annulations or rings is 40 or 41. As in some other species it is extraordinarily difficult to count their number. This is chiefly due to the fact that at both ends, but particularly at the anterior end, the lines demarcating the annulations are very faint and it is difficult to decide exactly where the first begins and where the last ends. Then the annulations do not appear to be true segments and no internal organs are serially homologous with them, so there is nothing by which to check the number. In *P. kachugensis* the annulations are confined to the ventral surface, they cease suddenly a little way up each side of the body and seen from the dorsal surface the animal is smooth and not ringed. (Pl. XX, fig. 7.)

The demarcating lines between the annulations are made more prominent by bearing a row of some 150—160 fine chitinous rods, somewhat sabre-shaped. These pierce the cuticle and externally end in sharp points projecting backward. (Pl. XX, figs. 9 and 10.) This is the first time I have seen such spines in a *Porocephalus*.

There is a slight median ventral groove which is shown well in Pl. XX, fig. 6.

The parasites were found encysted in the liver of a female *Kachuga lineata*, Gray, one of the Indian and Burmese representatives of the family Testudinidae. A piece of this organ, figured in Pl. XX, fig. 5, shows that there can have been but little of the tissue of the liver left; but this drawing represents the edge of the liver, the only part sent to me. The deeper parts of the liver may not have been so heavily infected.

(v) *POROCEPHALUS CLAVATUS*, Lohrmann.

A few specimens of Pentastomids were sent to me in February 1909 by Mr J. H. Ashworth of Edinburgh University taken in Northern Nigeria from the lung of the lizard, *Varanus exanthematicus* (= *V. ocellatus*). Three of these I take to be specimens of Lohrmann's species *Porocephalus clavatus* which has also been recorded from the lungs of *Varanus niloticus*.

(vi) *POROCEPHALUS BIFURCATUS*, Diesing.

The remaining specimens, of which there were two, with a fragment of a third, belong to what I take to be *Porocephalus bifurcatus*, Diesing. The latter species is not very fully described so I add a few particulars.

One specimen measured 21·5 mm. long and 1·5—2 mm. broad, the

other was much smaller and measured 9 mm. long and 1 mm. broad. The fragment was the same breadth as the bigger specimen and about two-thirds as long. The body is slightly curved.

The skin is extremely transparent and thrown in a thousand puckers and furrows which gives the animal a "bubbly" sort of facies. These puckers make it impossible to count the anterior rings. I should estimate the number of rings as about forty in number as in some specimens I examined in Vienna some years ago. The last is a long one and is preceded by some four or five which remain clearly recognizable. The form of the chitinous ring surrounding the mouth and of the hooks is very characteristic. The hooks are borne on projections like little tumuli which stand out from the head. These specimens also came from the lungs of *Varanus exanthematicus*, a new host for the species.

In conclusion it is worth mentioning that von Linstow has recently described a new species of *Porocephalus*, *P. indicus*, von Lins., from the trachea and lungs of a Gharial (*Gavialis gangeticus*, Geoffr.) which died in the Zoological Gardens, Calcutta.

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EXPLANATION OF PLATE XX.

Fig. 1. *Linguatula subtriquetra*, Diesing, × 2·5. Dorsal view.

Fig. 2. The same, × 2·5. Anterior view, showing the well-marked marginal flanges and the four anterior or pre-oral papillae.

Fig. 3. The same, × 2·5. Ventral view.

Fig. 4. The same, × 15. Ventral view of anterior end showing the hooks, the mouth and the two posterior or post-oral papillae.

Fig. 5. A piece of the edge of the liver of *Kachuga lineata*, × 1, showing intense infection with *Porocephalus kachugensis*.

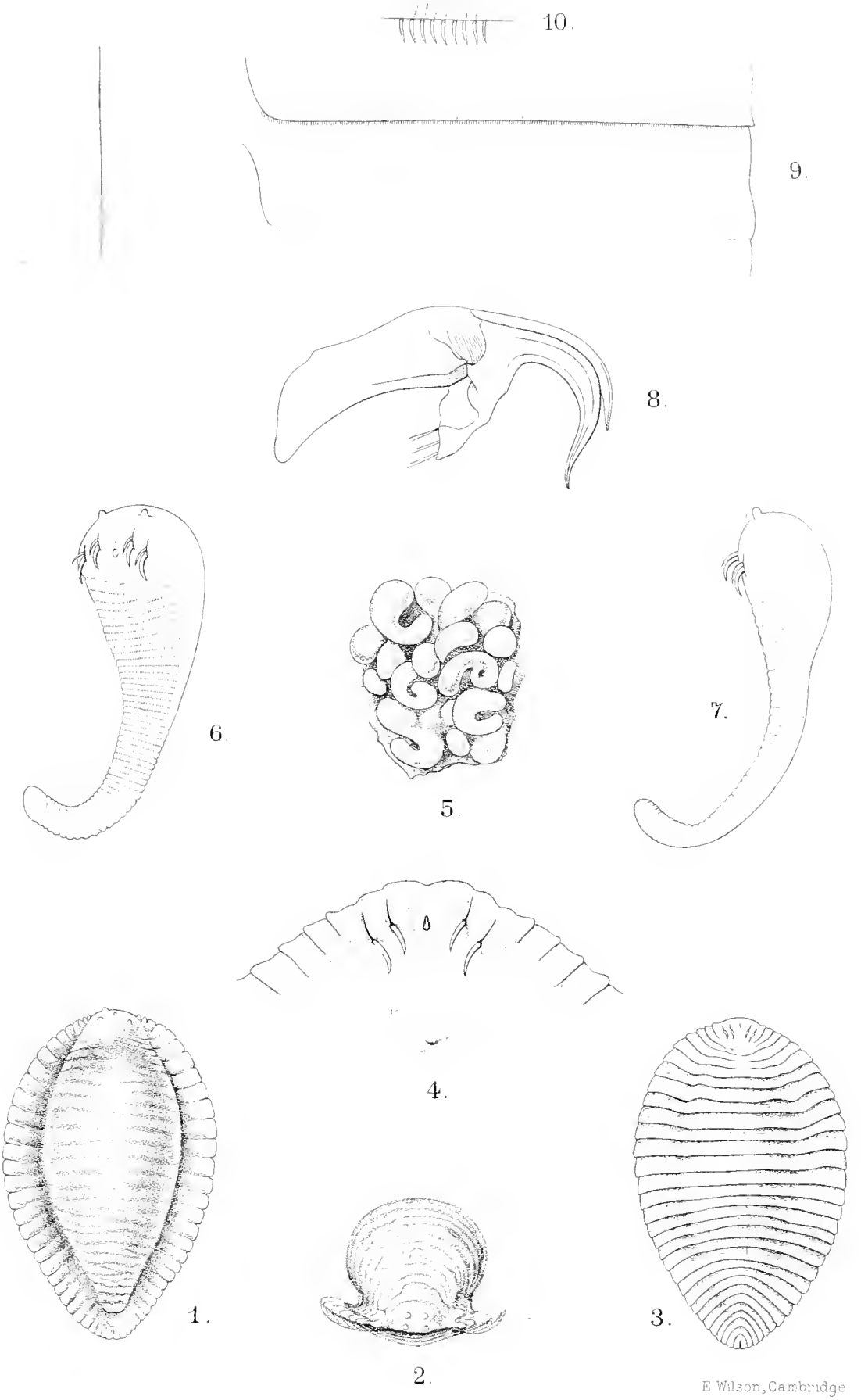
Fig. 6. *Porocephalus kachugensis*, × 4. Three-quarter view showing the mouth, four double hooks, the two conspicuous papillae, the incomplete annulations and the median ventral groove.

Fig. 7. *P. kachugensis*, × 4. Lateral view showing the hooks projecting unusually far, and the absence of annulation on the dorsal surface.

Fig. 8. Hook of *P. kachugensis*, × 30, showing the shaft and double hook.

Fig. 9. A portion of cuticle of *P. kachugensis* showing the arrangements of the sabre-shaped spicules between the annulations.

Fig. 10. The same spicules more highly magnified.



E Wilson, Cambridge

ON THE SO-CALLED BOTTLE-BACILLUS (*DERMATOPHYTON MALASSEZ*).

By HERMANN DOLD, M.D.

(With Plate XXI.)

The organism, commonly known under the name of the Bottle-bacillus (Flaschen-bacillus; Bacille-bouteille), was first described by Malassez (1874, pp. 203 et seq.), whose observations led him to the following conclusions (p. 211): "Il existe dans la pelade un champignon parasite. Ce champignon occupe les parties les plus superficielles de la couche cornée de l'épiderme; on le trouve entre ou à la surface des cellules épithéliales de cette couche. Il ne se rencontre qu'accidentellement sur les cheveux, et encore siége-t-il sur des cellules épithéliales, qui proviennent de l'épiderme cutané. Il est uniquement constitué par des spores sphériques très-petites. On peut en distinguer trois types:

"(1) Les premières mesurent de 4μ à 5μ, ont un double contour, peuvent avoir des bourgeons; ce sont les grosses spores.

"(2) Les seconds mesurent de 2μ à $2{,}5\mu$, n'ont pas de double contour, peuvent avoir des bourgeons; ce sont les petites spores.

"(3) Les troisièmes ont un diamètre inférieur à 2μ, un contour simple, pas des bourgeons; ce sont les sporules.

"Les spores ovoides que l'on peut encore rencontrer ne sont pas spéciales à la pelade et paraissent appartenir à une autre espèce de champignon.

"Il n'existe pas de tubes, mais seulement des petits chaplets, de 5μ à 6μ spores et plus. Ces résultats confirment la découverte de M. Gruby (*i.e. Microsporon audouini*), dans ce qu'elle a d'essentiel; mais ils en diffèrent complètement sur quelques points de détail."

Thus it is evident that Malassez had before him the same organism which was subsequently described by Unna (1894), and called "Flaschen-bacillus" (= Bottle-bacillus), because of the bottle- or flask-shaped form which it shows under natural conditions on the skin. This is the name

by which the organism is commonly known. It will also be noted that Malassez distinguishes three types of the organism, a distinction which is based on the size of the "spores"; further that he believes that the ovoid forms which he also observed have nothing to do with the "champignon de la pelade" but belong to a special kind of parasite. He also thought it necessary to discuss the question of the identity or not of his "champignon de la pelade" with the parasite previously detected by Gruby (1843), the "*Microsporon audouini*."

In another paper Malassez (1874 (*a*), p. 451) describes the parasite found in Pityriasis, which, although having a great resemblance to the "champignon de la pelade," differs therefrom, in his opinion, in some points, for he states (p. 463) that "Ils se distinguent de celles de la pelade en ce que ces dernières sont habituellement sphériques et plus volumineuses ; de celles des autres champignons connus en ce que ces dernières possèdent des tubes de mycélium."

Although the organism has been more fully described by Unna (1894), and by Sabouraud (1897, 1902), there is, as yet, no unanimity as to the part it plays in pathology. Its discoverer, Malassez (1874 (*a*), p. 463), was inclined to see in the "champignon de la pityriasis simple" the cause of the condition : "Ils paraissent jouer dans la pathogénie du pityriasis le même rôle que les autres parasites dans celles des affections cutanées généralement considerées comme de nature parasitaire."

I cannot find a definite statement in Unna's papers of his view regarding the part played by this organism, but it seems to me, that he considers it to be a mere saprophytic inhabitant of the skin. Sabouraud, on the other hand, has apparently changed his former view on the "Bacille-bouteille." In his first paper (1897) he says, "On peut rencontrer accidentellement une dizaine d'espèces microbiennes différentes, parmi lesquelles deux sont constantes. Ce sont le Bacille-bouteille de Unna,.....et qui paraît n'avoir aucune valeur pathogène." In his second publication, when speaking about the relationship between the Bottle-bacillus and Pityriasis, he says, "Et sa présence aux points mêmes où l'on voit les couches épidermiques se cliver, semble affirmer son rôle causal dans le clivage même et l'exfoliation de l'épiderme."

Whitfield (1907) also seems inclined to ascribe a pathogenic rôle to the bottle-bacillus, in Pityriasis: "No one can work at the distribution of Pityriasis alba, and the Bottle-bacillus, without being struck by their invariable association," whereas Malcolm Morris (1908) considers that "the rôle played by the Bottle-bacillus requires further elucidation."

Frequent trials have been made to obtain this organism in pure culture, but apparently without success. Possibly both Unna[1] and Whitfield[2] had at one time, without realising the fact, a pure culture of the organism, but it is of course impossible now to be certain about it.

As I announced in my preliminary note (1909), I have succeeded in obtaining pure cultures of the bottle-bacillus. Since the appearance of my publication, I have made a study of the morphological and biological characters of this organism, which, for reasons given in the course of this paper, I shall henceforth refer to as *Dermatophyton Malassez* (or "D. M." for brevity).

Occurrence of the Dermatophyton Malassez.

As shown by Unna, this organism is found in many conditions of the skin. It is present in larger or smaller numbers, alone or associated with other bacteria, in Rosacea seborrhoica, Pityriasis capitis, Alopecia pityroides, Alopecia seborrhoica, Alopecia excematosa, Pityriasis rubra seborrhoica, and in Comedones. The organism is also almost invariably found in apparently healthy persons, in horny cells and the sebum of the skin, especially of the scalp.

There is certainly a great variation in the number of D. M. to be met with on individual skins. The more a skin inclines to a seborrhoic and pityroid state, the more abundantly are the organisms present, but in examining a large number of scalps, I did not come across one case, where the presence of D. M. could not be demonstrated. I might mention here, that in animals (Rats and Rabbits), I did not encounter the organism; my examinations in this direction are however too few to justify a definite statement.

Methods.

In examining for the D. M. it is perhaps best to use scrapings from the scalp. These may easily be obtained, by drawing the edge of a slide over the scalp, at the same time pressing the slide firmly against

[1] Unna (1894), pp. 233 and 234, says "An abgekratzten Hornzellen habe ich microscopisch öfters grössere Mengen der Flaschenbacillen constatiert, doch die Culturen ergaben gleichzeitig Morokokken."

[2] Whitfield (1907, p. 76) states that "The organism has not been grown in pure culture, but on one occasion I succeeded in getting an impure culture, which however rapidly died out."

the skin. The material thus obtained is smeared on the slide. The smear is fixed by passing it through a flame, after which it is further fixed, and freed from grease, by immersing the slide, in equal parts of alcohol and ether, for 5 minutes. The smear may now be stained with any aniline dye. In stained specimens the D. M. is found as larger or smaller spheroid or ovoid bodies, which very often show a protrusion or budding at one end, like yeast cells. These bodies represent flask- or bottle-like forms (see Plate XXI, fig. 1). Here and there are found bacillary forms of varying length and thickness, filaments, and short chains of the above-mentioned round bodies.

The Cultures.

For the cultivation of this organism the material obtained in the way above described is ground up and emulsified in 0·5 to 1·0 c.c. of sterile normal saline solution or broth, and then plated out on ordinary nutrient agar, or, preferably, on acid agar. The plates are then incubated at 37° C. The colonies of D. M. become visible to the naked eye in 24 hours as delicate round discs, dull white by reflected light, pearl-grey by transmitted light. If examined with a low power, the colony appears finely granular, the margin being slightly serrated. As the colony grows older, the margin becomes more and more irregular. Stained preparations of such a colony show large coccus-like forms, partly arranged in short chains, partly in clusters, so that at the first glance they might be taken for Staphylococci (see Plate XXI, fig. 2). A more careful observation shows, however, some differences. First of all the forms are, as a whole, somewhat large for Staphylococci; here and there a much smaller cell adheres to a larger one, like a daughter to a mother cell. Intermixed with these coccus forms, there are ovoid and short bacillary forms, the latter being rarely encountered. Viewed in a hanging drop preparation, the organisms are not motile; they contain granules, vacuoles, and many have a more polyhedric than exactly round form.

If the same organism be subcultured on an acid medium, for example Sabouraud's, recommended for the cultivation of *Bacillus acnes* (agar 15 grms.; peptone 20 grms.; glycerine 20 grms.; distilled water 1 litre; glac. acetic acid 5 drops) the growth is far more abundant. A stained preparation shows very irregular, but as a whole much larger forms than those grown on ordinary agar. The organisms appear as large round bodies, some swollen and faintly stained (involution forms ?), others as

large ovoid and bacillary forms, some budding, some showing irregular swellings along their course, others again being club-shaped (see Plate XXI, fig. 3).

On other solid media, such as ordinary serum, they grow in much the same way as on agar. They do not change the colour of neutral-red agar, and form a light red shining film on the top of the stab culture. On potato at 22° C. the organisms form within two to three days a moist shining yellowish film, which spreads slowly. Potato cultures at 37° C. scarcely show any growth.

The growth of this organism in gelatin stab cultures is most characteristic. Two or three days after inoculation the organisms grow in a fine line along the track of the needle, and form a delicate bluish or yellowish white film on the top. After from one to two weeks, a short radiating outgrowth appears along the track of the needle, whereas the growth on the top represents a sort of "corona radiata," with a central shallow pit. The organisms continue to grow slowly in this regularly radiating manner on the top, and, after 3 or 4 weeks, the single "radii," starting from the periphery, split up into separate segments or leaflets, so that the appearance of a "Daisy-Head" results (see Plate XXI, fig. 5). The separate leaflets soon cease to grow with the same regularity as hitherto and in cultures two to four months old, the growth on the surface of the gelatin is somewhat fernlike.

In broth, the D. M. develops still more peculiar and irregular forms than on acid media, the most remarkable feature being the development of long filaments and threads; besides there are large spheroid and ovoid forms, with or without budding, and chains of large round bodies. Sometimes a sort of false branching of the filaments can be seen, but I also observed instances of true branching (see Plate XXI, fig. 4). The broth shows in 24—28 hours after inoculation a general turbidity, but later on clears up, a deposit being formed at the bottom. If the organisms be again subcultured on ordinary solid media (agar, serum, etc.) the coccoid forms reappear (as in Plate XXI, fig. 2).

The same growth as in broth occurs in other liquid media, peptone water, milk, etc.

Milk remains unchanged. Indol is not formed.

The organisms grow aerobically and anaerobically, either at 22° C. or 37° C.

Staining reactions.

The organisms are not acid-fast. When stained according to Gram's method, they are as a rule found to be Gram-positive; the larger forms of the organism (bacilli, filaments) are however often decolourised.

Fermentation reactions.

As the morphological characters of the D. M. seemed to suggest a yeast-like nature, I thought it worth while to test its effect upon a series of sugars, and upon starch. The results of these experiments are given in the following table.

Observations made three weeks after inoculation.

Sugars	Acid	Gas	Remarks
Glucose	+	+	—
Lactose	(+)	–	Only traces of acid.
Galactose	–	–	—
Levulose	(+)	–	Only traces of acid.
Maltose	(+)	–	Only traces of acid.
Raffinose	–	–	—
Cane-sugar	–	–	—
Dulcite	–	–	—
Mannite	–	–	—
Inuline	–	–	—
Starch	(+)	–	Only traces of acid.

A glance at the above table shows that the D. M. has not great fermentative power, glucose being the only sugar that shows a distinct though rather slow fermentation, the production of acid not being recognizable until the second or third day. The amount of acid and gas formed in a three weeks' culture in glucose broth is inconsiderable.

Pathogenicity.

I experimented on eight animals, two rats, two guinea-pigs, and four rabbits. The two rats were shaved on the back, over an area of about two inches in diameter, any injury of the skin being carefully avoided. Material from a two days' broth culture was then rubbed into the clean

shaven skin. No pathogenic effect followed. The shaven part of the skin again became covered with hair. The skin and hair both remained normal.

The two guinea-pigs were each inoculated intraperitoneally with 0·5 c.c. of a two days' broth culture. The animals showed a transitory indisposition, but soon recovered, and remained healthy for four months (present date).

Two rabbits were injected subcutaneously in the back, with 0·5 c.c. and 1·0 c.c. respectively, of a three days' culture of the organism. No suppuration resulted. The organisms were evidently destroyed and absorbed. The animals remained healthy for four months (present date).

Two other rabbits received respectively 0·5 and 1·0 c.c. of a three days' broth culture injected into the ear-vein. After a period of uneasiness, lasting one to two days, the animals recovered and seemed healthy. They were killed three months after the inoculation. The organs were normal, excepting one suprarenal gland; this was enlarged, and was found on microscopic sections to contain suppurating foci, in which numerous D. M. (spheroid, ovoid, bacillary and bottle forms) were present.

From these experiments it is evident that the D. M. is not pathogenic for rats and guinea-pigs. The slight effect observed in one rabbit, after the intravenous injection of a large dose of culture, is curious, but cannot be accepted as sufficient evidence that the organism is really pathogenic. The experiments on animals do not of course exclude its being pathogenic to man, though this appears rather unlikely; I regret that I had no opportunity of testing this experimentally.

Malassez distinguishes three types of this organism, the distinction being based on the size of the individual cells. Having shown the extraordinary polymorphism, of which D. M. is capable, I think that this distinction is not justified. The same is true of his distinction between the "champignon de la pelade" and the "champignon de la pityriasis simple" and of his assumption that the ovoid forms to be met with belong to another species. The mere morphological differences, which led Malassez to regard these forms as distinct, also appear to me to be insufficient. It is possible that the organisms found in Pityriasis differ biologically from those found on healthy or seborrhoic skins (like those from which I obtained my cultures), but it is most improbable.

Conclusions.

It appears, therefore, that the organism described in this paper is a harmless permanent inhabitant of the superficial layer of the human skin, and that its presence in larger or smaller number depends on the more or less favourable conditions existing in different skins. Diseased skins, especially seborrhoic and pityroid, offer evidently the best conditions for the existence and multiplication of this organism.

The greasy state of the skin in Seborrhoea, the larger amount of horny cells thrown off in Pityriasis, the increased disintegration on the surface of the skin, together with an increased formation of fatty acids, may be regarded as conditions which favour the growth of this saprophyte. I would revert here to the fact that the organism showed a far more abundant growth in acid than in alkaline or neutral media.

Taking into consideration the cultural results obtained, I believe (contrary to my opinion expressed in my preliminary note) that the organism should rather be placed between the Hyphomyceta and Blastomyceta, than classified with the latter.

It appears to belong to the genus *Oidium*, together with the other well-known parasites of the skin.

The name "*Bottle-bacillus*," though very well expressing a morphological characteristic of this organism, is inadequate, if one considers its position in the system of bacteria. Malassez, when he first described this organism, called it a "champignon," and thought it closely related to the *Microsporon audouini* of Gruby. My experiments seem to corroborate Malassez's original view, in so far as they indicate that this organism, though evidently a rather harmless saprophyte of the skin, and of the skin only, belongs to the same group as the pathogenic parasite of skin and hairs (*Trichophyta*); I therefore venture to propose for this organism the name *Dermatophyton Malassez*.

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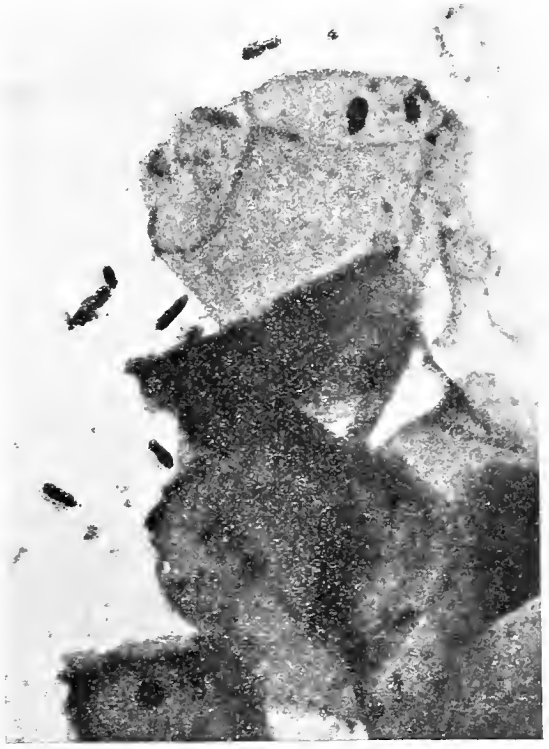

Fig. 1.

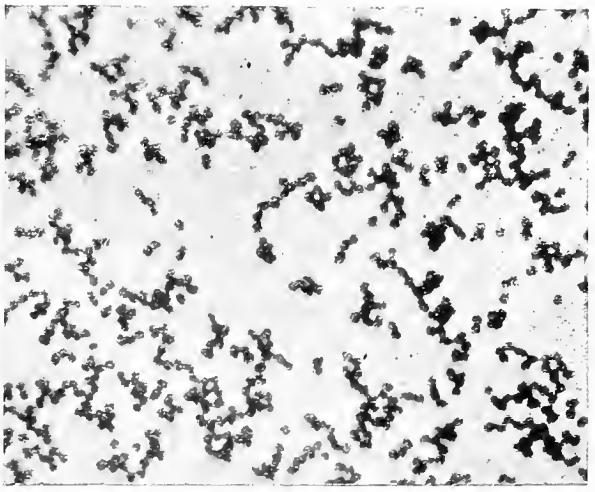

Fig. 2.

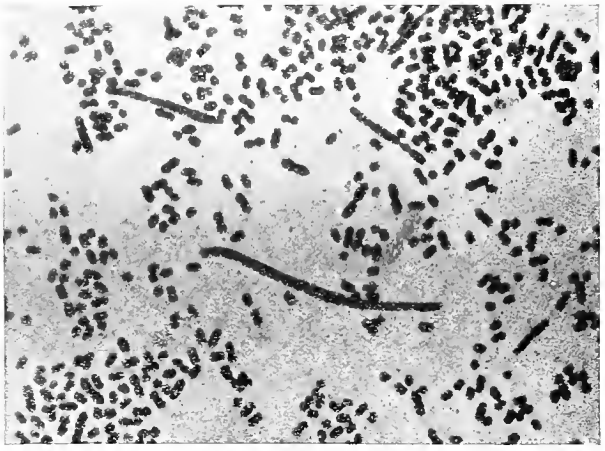

Fig. 3.

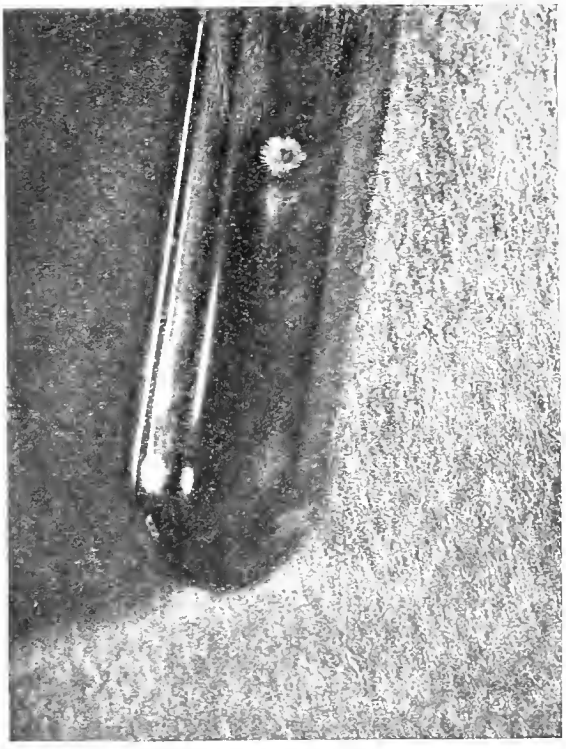

Fig. 5.

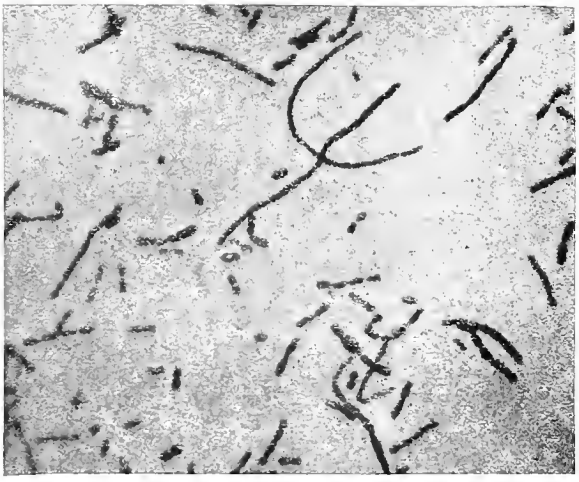

Fig. 4.

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EXPLANATION OF PLATE XXI.

The photo-micrographs were all taken with Leitz oil immersion objective $\frac{1}{12}$, and ocular 4. Stained with Fuchsin.

Fig. 1. Scrapings of seborrhoic scalp, showing scratched-off horny cells, and *Dermatophyton Malassez* (large round cells, bacillary and bottle forms). ×1200.

Fig. 2. Two days' culture of *D. Malassez* on ordinary agar, showing coccoid forms. ×1200.

Fig. 3. Two days' culture of *D. Malassez* on acid agar (Sabourand's medium), subcultured from an ordinary culture on agar (Fig. 2) showing large, round, ovoid forms, bacilli, and filaments with spindle-like swellings. Near the centre two large ovoid forms, faintly stained. ×1200.

Fig. 4. Two days' culture of *D. Malassez*, in ordinary broth, subcultured from ordinary agar culture (Fig. 2), showing large round and ovoid forms, bacilli, "bottles," and filaments. ×1200.

Fig. 5. Four weeks' gelatin stab culture of *D. Malassez* showing the radiating outgrowth along the needle track, and the "Daisy-head" on the top. ×$1\frac{1}{2}$.

THE CURE OF SURRA IN HORSES BY THE ADMINISTRATION OF ARSENIC.

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(With Plates XXII to XXVII.)

In a previous paper which appeared in *Parasitology*, Vol. III. pp. 73—107, and the *Journal of Tropical Veterinary Science*, Vol. II. No. I, details were given of thirty-two cases of successful treatment of Surra in horses by means of arsenic and its derivatives.

The object of the present article is to report on the progress of these cases under a prolonged period of observation, and to describe further experiments which have been carried out, on the same lines of treatment, in the Muktesar Laboratory.

Both results further confirm the conclusion, previously arrived at, that the treatment effects a complete cure of Surra, and not merely establishes a temporary tolerance of the disease. In the former publication, a tabulated list was given of Surra animals which had shown no return of the disease after treatment, together with the periods during which they had been kept under daily observation (*Parasitology*, Vol. III. p. 103).

These periods were dated up to September 30th, 1909. Since that date these animals have been at work, and their blood has been examined at intervals of two and three weeks.

The last examination was made on Jan. 31st, 1910, at which time all were in good condition, and no trypanosomes could be detected in their blood. During this period of four months (Oct. 1909 to Jan. 1910) a number of dogs, rabbits and guinea-pigs were inoculated with blood from these animals. All gave negative results. In February 1910 I left India, on furlough, but arranged that a number of these Surra recovered ponies will be available for examination on my return, at the end of the present year.

The period of observation for each of these thirty-two cases, dated to Jan. 31st, 1910, is as follows:

No. of animal	Day of disease	Paroxysm	Drugs used	Period under observation
Horse C 1	17th	3rd	ars. ac. alone	10 months
,, C 2	19th	3rd	,,	10 ,,
Pony C 3	25th	4th	,,	7½ ,,
,, C 4	20th	3rd	,,	7 ,,
,, C 5	6th	1st	,,	12 ,,
,, C 6	19th	3rd	,,	14½ ,,
,, C 7	6th	1st	,,	9¼ ,,
,, C 8	14th	1st	,,	7¼ ,,

(*1st method of dosage.*)

The atoxyl and arsenic administered alternately leaving one day interval between each dose.

No. of animal	Day of disease	Paroxysm	Drugs used	Period under observation
Mule C 9	13th	2nd	Atoxyl & ars. ac.	11½ months
,, C 10	12th	2nd	,,	11½ ,,
Pony C 11	5th	...	,,	8 ,,
,, C 12	4th	1st	,,	14 ,,
,, C 13	5th	1st	,,	14 ,,
,, C 14	1st	1st	,,	18 ,,
,, C 15	5th	1st	,,	16 ,,
,, C 16	1st	1st	,,	16 ,,
Horse C 17	Spontaneous case in advanced stage	—	,,	16 ,,

(*2nd method of dosage.*)

A dose of arsenious acid in solution (Loeffler and Ruehs) followed on consecutive days by a dose of atoxyl (subcutaneously) and a dose of arsenious acid in bolus.

Pony	C 18	4th	1st	Atoxyl & ars. ac.	11½ months
,,	C 19	18th	3rd	,,	13 ,,
,,	C 20	20th	4th	,,	13½ ,,
,,	C 21	5th	1st	,,	14 ,,
,,	C 22	8th	2nd	,,	12 ,,
,,	C 23	13th	2nd	,,	13 ,,
,,	C 24	13th	2nd	,,	13 ,,
,,	C 25	13th	2nd	,,	13 ,,
,,	C 26	13th	2nd	,,	13 ,,
,,	C 27	13th	2nd	,,	13 ,,
,,	C 28	13th	2nd	,,	12½ ,,
,,	C 29	2nd	1st	,,	15 ,,
,,	C 30	21st	3rd	arsacetin	12½ ,,
,,	C 31	22nd	3rd	soamin	12½ ,,
,,	C 32	24th	2nd	atoxyl, tartar emetic and ars. ac.	16 ,,

Small animals inoculated with blood from the Surra treated cases under observation.

Dog (1). Inoculated intraperitoneally with 20 c.c. citrated blood from Pony C 6. Blood examined daily for six weeks. Result negative.

Dog (2). Inoculated intraperitoneally with 25 c.c. defibrinated blood from Pony C 16. Blood examined daily for six weeks. Result negative.

Dog (3). Inoculated intraperitoneally with 10 c.c. fresh blood from Pony C 12. Blood examined daily for six weeks. Result negative.

Dog (4). Inoculated intraperitoneally with 30 c.c. citrated blood from Horse C 17. Blood examined daily for six weeks. Result negative.

Dog (5). Inoculated subcutaneously with 5 c.c. fresh blood from Pony C 29. Blood examined daily for six weeks. Result negative.

Dog (6). Inoculated subcutaneously with 10 c.c. citrated blood from Pony C 14. Blood examined daily for six weeks. Result negative.

Dog (7). Inoculated subcutaneously with 20 c.c. citrated blood from Pony C 24. Blood examined daily for six weeks. Result negative.

Twelve rabbits and the same number of guinea-pigs were inoculated subcutaneously with fresh blood varying in amounts from 1 c.c. to 5 c.c. from ten of the treated cases. Blood was examined daily for six weeks. Results negative.

Further experiments in the treatment of Surra.

With a view to further confirm the results already obtained in the treatment of Surra by means of arsenic alone, and arsenic in combination with atoxyl; and to test the treatment on mules, a number of ponies and mules were inoculated with Surra in July 1909. When the disease was well established, treatment was carried out by methods of dosage similar to those adopted in the previous experiments.

Nine mules and six ponies were subjected to treatment by the 2nd or combined method (a dose of arsenious acid in solution—Loeffler and Ruehs—followed on consecutive days by a dose of atoxyl subcutaneously and a dose of arsenious acid in bolus). From this number seven mules and four ponies recovered.

Three ponies and two mules were treated with arsenic alone. One mule and two ponies recovered. It was intended to subject a larger number of animals to this method of treatment, but at the time no more animals were available for the experiment.

Twelve ponies were treated with atoxyl and arsenious sulphide chemically pure; one pony recovered.

Treatment with atoxyl and arsenic.

A four per cent. solution of atoxyl freshly prepared was used. The solution of arsenic was made according to the method of Loeffler and Ruehs as follows:

Take 1 gramme arsenious acid, 10 c.c. normal soda solution. Dissolve by boiling. Add 10 c.c. normal hydrochloric acid and make up to 1000 c.c.

Normal soda solution:

42 grammes pure caustic soda. Dissolve in 800 c.c. aq. dist. When dissolved make up to 1000 c.c.

Normal hydrochloric acid solution:

Take 181 grammes (weight) hydrochloric acid @ 1·1 sp. g. and make up to 1000 c.c.

Mule C 33. 530 lbs., 2nd paroxysm. 15th day of disease.

1st day 70 c.c. atoxyl.
2nd ,, 500 c.c. ars. ac. sol.
3rd ,, 1 gm. ars. ac.
Six days interval.
10th day 500 c.c ars. ac. sol.
11th ,, 60 c.c. atoxyl.
12th day 1·5 gms. ars. ac.
Four days interval.
17th day 800 c.c. ars. ac. sol.
18th ,, 60 c.c. atoxyl.
19th ,, 2·5 gms. ars. ac.

No return of trypanosomes. Kept under observation 5 months.

Mule C 34. 550 lbs., 3rd paroxysm. 25th day of disease.

1st day 60 c.c. atoxyl.
3rd ,, 500 c.c. ars. ac. sol.
4th ,, 60 c.c. atoxyl.
5th ,, 1 gm. ars. ac.
Four days interval.
10th day 500 c.c. ars. ac. sol.
11th ,, 60 c.c. atoxyl.
12th ,, 1·5 gms. ars. ac.
Four days interval.
17th day 600 c.c. ars. ac. sol.
18th ,, 60 c.c. atoxyl.
19th ,, 2 gms. ars. ac.
Eight days interval.
28th day 600 c.c. ars. ac. sol.
29th ,, 60 c.c. atoxyl.
30th ,, 2 gms. ars. ac.

No return of trypanosomes. Kept under observation 5 months.

Mule C 35. 500 lbs., 2nd paroxysm. 15th day of disease.

1st day 60 c.c. atoxyl.
3rd ,, 600 c.c. ars. ac. sol.
4th ,, 60 c.c. atoxyl.
5th ,, 1·5 gms. ars. ac.
Four days interval.
10th day 800 c.c. ars. ac. sol.
11th ,, 60 c.c. atoxyl.
12th ,, 2 gms. ars. ac.
Four days interval.
17th day 800 c.c. ars. ac. sol.
18th ,, 50 c.c. atoxyl.
19th ,, 2 gms. ars. ac.
Eight days interval.
28th day 800 c.c. ars. ac. sol.
29th ,, 50 c.c. atoxyl.
30th ,, 2 gms. ars. ac.

No return of trypanosomes. Kept under observation 5 months.

Mule C 36. 500 lbs., 3rd paroxysm. 25th day of disease.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Four days interval.
8th day 500 c.c. ars. ac. sol.
9th ,, 60 c.c. atoxyl.
10th ,, 1·5 gms. ars. ac.
Four days interval.
15th day 1000 c.c. ars. ac. sol.
16th ,, 60 c.c. atoxyl.
17th ,, 2 gms. ars. ac.
Eight days interval.
26th day 800 c.c. ars. ac. sol.
27th ,, 50 c.c. atoxyl.
28th ,, 2 gms. ars. ac.

No return of trypanosomes. Kept under observation 5 months.

Mule C 37. 570 lbs., 3rd paroxysm. 25th day of disease.

1st day 50 c.c. atoxyl.
3rd ,, 400 c.c. ars. ac. sol.
4th ,, 50 c.c. atoxyl.
5th ,, 1 gm. ars. ac.
Four days interval.
10th day 400 c.c. ars. ac. sol.
11th ,, 50 c.c. atoxyl.
12th ,, 1 gm. ars. ac.
Four days interval.
17th day 1000 c.c. ars. ac. sol.
18th ,, 60 c.c. atoxyl.
19th ,, 2 gms. ars. ac.
Eight days interval.
28th day 800 c.c. ars. ac. sol.
29th ,, 50 c.c. atoxyl.
30th ,, 2·5 gms. ars. ac.

No return of trypanosomes. Kept under observation 5 months.

Mule C 38. 550 lbs., 3rd paroxysm. 25th day of disease.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Six days interval.
10th day 600 c.c. ars. ac. sol.
11th ,, 60 c.c. atoxyl.
12th day 1·5 gms. ars. ac.
Four days interval.
17th day 1000 c.c. ars. ac. sol.
18th ,, 60 c.c. atoxyl.
19th ,, 2·5 gms. ars. ac.

No further treatment was given as the animal showed symptoms of arsenic poisoning after the last dose. No return of trypanosomes. Kept under observation 5 months.

Mule C 39. 460 lbs., 2nd paroxysm. 15th day of disease.

1st day 600 c.c. ars. ac. sol.
2nd ,, 50 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Four days interval.
8th day 700 c.c. ars. ac. sol.
9th ,, 50 c.c. atoxyl.
10th day 1·5 gms. ars. ac.
Eight days interval.
19th day 800 c.c. ars. ac. sol.
20th ,, 50 c.c. atoxyl.
21st ,, 2 gms. ars. ac.

No return of trypanosomes. Kept under observation 5 months.

Mule B 1. 660 lbs., 3rd paroxysm. 25th day of disease.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Four days interval.
8th day 500 c.c. ars. ac. sol.
9th ,, 50 c.c. atoxyl.
10th ,, 1 gm. ars. ac.
Four days interval.
15th day 600 c.c. ars. ac. sol.
16th ,, 70 c.c. atoxyl.
17th ,, 1·5 gms. ars. ac.
Eight days interval.
26th day 800 c.c. ars. ac. sol.
27th ,, 50 c.c. atoxyl.
28th ,, 2 gms. ars. ac.

Trypanosomes reappeared 17 days after treatment. In this case the doses were not sufficiently large. The amounts were not increased, as the animal showed symptoms of poisoning after the first series of doses. A second course of treatment with larger amounts would probably have succeeded. This was not tried owing to the number of other experiments in hand at the time.

Mule B 2. 350 lbs., 3rd paroxysm. 25th day of disease.

1st day 50 c.c. atoxyl.
3rd ,, 400 c.c. ars. ac. sol.
4th ,, 50 c.c. atoxyl.
5th ,, 1 gm. ars. ac.
Eight days interval.
14th day 400 c.c. ars. ac. sol.
15th day 50 c.c atoxyl.
16th ,, 1·5 gms. ars. ac.
Four days interval.
21st day 600 c.c. ars. ac. sol.
22nd ,, 60 c.c. atoxyl.
23rd ,, 2 gms. ars. ac.

This mule showed symptoms of poisoning on the 25th day and died that night.

Pony C 40. 300 lbs., 3rd paroxysm. 20th day of disease.

1st day 50 c.c. atoxyl.
3rd ,, 400 c.c. ars. ac. sol.
4th ,, 50 c.c. atoxyl.
5th ,, 1 gm. ars. ac.
Four days interval.
10th day 400 c.c. ars. ac. sol.
11th ,, 50 c.c. atoxyl.
12th ,, 1 gm. ars. ac.
Four days interval.
17th day 500 c.c. ars. ac. sol.
18th ,, 60 c.c. atoxyl.
19th ,, 1·5 gms. ars. ac.
Four days interval.
24th day 600 c.c. ars. ac. sol.
25th ,, 60 c.c. atoxyl.
26th ,, 1 gm. ars. ac.

No return of trypanosomes. Kept under observation 5 months.

Pony C 41. 400 lbs., 3rd paroxysm. 20th day of disease.

1st day 500 c.c. ars. ac. sol.
2nd ,, 50 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Four days interval.
8th day 500 c.c. ars. ac. sol.
9th ,, 50 c.c. atoxyl.
10th day 1 gm. ars. ac.
Eight days interval.
19th day 600 c.c. ars. ac. sol.
20th ,, 50 c.c. atoxyl.
21st ,, 1·5 gms. ars. ac.

No return of trypanosomes. Kept under observation 5 months.

Pony C 42. 550 lbs., 3rd paroxysm. 20th day of disease.

1st day 600 c.c. ars. ac. sol.
2nd ,, 50 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Four days interval.
8th day 600 c.c. ars. ac. sol.
9th ,, 50 c.c. atoxyl.
10th ,, 1 gm. ars. ac.
Six days interval.
17th day 700 c.c. ars. ac. sol.
18th ,, 60 c.c. atoxyl.
19th ,, 2 gms. ars. ac.
Eight days interval.
28th day 600 c.c. ars. ac. sol.
29th ,, 60 c.c. atoxyl.
30th ,, 1·5 gms. ars. ac.

No return of trypanosomes. Kept under observation 5 months.

Pony C 43. 350 lbs., 3rd paroxysm. 20th day of disease.

1st day 50 c.c. atoxyl.
3rd ,, 500 c.c. ars. ac. sol.
4th ,, 50 c.c. atoxyl.
5th ,, 1 gm. ars. ac.
Six days interval.
12th day 400 c.c. ars. ac. sol.
13th ,, 50 c.c. atoxyl.
14th ,, 1 gm. ars. ac.
Four days interval.
19th day 600 c.c. ars. ac. sol.
20th ,, 50 c.c. atoxyl.
21st ,, 1·5 gms. ars. ac.
Eight days interval.
30th day 600 c.c. ars. ac. sol.
31st ,, 50 c.c. atoxyl.
32nd ,, 1·5 gms. ars. ac.

No return of trypanosomes. Kept under observation 5 months.

Pony B 3. 400 lbs., 3rd paroxysm. 20th day of disease.

1st day 400 c.c. ars. ac. sol.
2nd ,, 50 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Four days interval.
8th day 500 c.c. ars. ac. sol.
9th ,, 50 c.c. atoxyl.
10th ,, 1 gm. ars. ac.
Six days interval.
17th day 600 c.c. ars. ac. sol.
18th ,, 60 c.c. atoxyl.
19th ,, 1 gm. ars. ac.
Eight days interval.
28th day 500 c.c. ars. ac. sol.
29th ,, 60 c.c. atoxyl.
30th ,, 1·5 gms. ars. ac.

Trypanosomes reappeared 22 days after treatment.

Pony B 4. 350 lbs., 3rd paroxysm. 20th day of disease.

1st day 50 c.c. atoxyl.
3rd ,, 500 c.c. ars. ac. sol.
4th ,, 50 c.c. atoxyl.
5th ,, 1 gm. ars. ac.
Four days interval.
10th day 600 c.c. ars. ac. sol.
11th ,, 60 c.c. atoxyl.

This pony showed symptoms of poisoning on the 12th day and died the same night.

Treatment with arsenic alone.

Mule C 44. 500 lbs., 2nd paroxysm. 18th day of disease.

1st day 3 gms. ars. ac.

Treatment was not continued as the mule showed symptoms of poisoning and was off feed for several days.

No return of trypanosomes. Kept under observation 5 months.

Mule B 5. 450 lbs., 2nd paroxysm. 18th day of disease.

1st day 1 gm. ars. ac.
3rd ,, 2 gms. ,,
6th ,, 3 ,, ,,

This mule showed symptoms of poisoning and died on the 8th day.

Pony C 45. 300 lbs., 2nd paroxysm. 18th day of disease.

1st day 1 gm. ars. ac.
3rd ,, 1 ,, ,,
5th ,, 1·5 gms. ,,
8th ,, 1·5 ,, ,,
10th day 1·25 gms. arc. ac.
12th ,, 2 ,, ,,
14th ,, 1·5 ,, ,,
16th ,, 1·25 ,, ,,

No return of trypanosomes. Kept under observation 5 months.

Pony C 46. 350 lbs., 2nd paroxysm. 18th day of disease.

1st day 1 gm. ars. ac.
3rd ,, 1 ,, ,,
5th ,, 1 ,, ,,
7th ,, 1·5 gms. ,,
9th ,, 1·75 ,, ,,
11th day 1·75 gms. ars. arc.
13th ,, 1·75 ,, ,,
15th ,, 1·5 ,, ,,
17th ,, 1·75 ,, ,,

No return of trypanosomes. Kept under observation 5 months.

Treatment with atoxyl and arsenious sulphide (chemically pure).

Twelve ponies were treated with atoxyl and arsenious sulphide. The results were unsatisfactory. Ponies of 500 lbs. weight do not tolerate more than 10 gms. of the sulphide. In many cases a smaller dose proves fatal. Even in small amounts it is not borne so well as the arsenious acid.

Out of the twelve ponies treated five were discontinued at an early stage as each dose was followed by symptoms of poisoning. In six cases the treatment was pushed as far as possible, but relapses occurred a few weeks after the dosage was stopped. In one case the treatment was successful.

Pony C 47. 350 lbs., 3rd paroxysm. 18th day of disease.

1st day 50 c.c. atoxyl.
3rd ,, 6 gms. ars. sulph.
5th ,, 50 c.c. atoxyl.
9th ,, 6 gms. ars. sulph.
11th day 50 c.c. atoxyl.
13th ,, 6 gms. ars. sulph.
15th ,, 50 c.c. atoxyl.

At this stage the pony showed symptoms of poisoning, and was in pain and off food for five days. No further treatment was given.

No return of trypanosomes. Kept under observation 5 months.

The result of the arsenic treatment in twenty cases of Surra spontaneously contracted.

In the Autumn of 1909 Surra broke out among the Army Transport ponies working on the Kathgodam road. Twenty of these cases were sent to the Laboratory for treatment. All these ponies were old, not less than sixteen years, worn out, and in the last stage of the disease. They were so debilitated that it required two days to march them from their camp to the Laboratory, a distance of about 18 miles.

On blood examination trypanosomes varying in number from 5 to 100 in a field were detected in every case. The animals were not in a condition to tolerate the administration of arsenic by the mouth. Consequently, an injection of atoxyl was first given which freed the blood from trypanosomes. The injection was repeated after an interval of three days. After a week the ponies had so far improved that it was considered safe to commence treatment. Out of these twenty cases, two died from tympanitis during the course of treatment. There were no symptoms, or post mortem lesions of poisoning, and death was not attributed to arsenic.

One pony died some weeks after the completion of treatment from injuries received by falling under the partition of his stall, at night. He had not shown any relapse.

Seventeen recovered. Four of these had a relapse and received a second course of treatment. Sixteen ponies were returned to their corps after being kept under observation at the Laboratory for three months following their treatment. One pony which injured his hock and is unfit for work remains under observation at the Laboratory.

Taking into consideration the age and condition of these animals, the fact that the disease was contracted spontaneously and had advanced to the last stage, it is evident, that, no more severe or practical test could be applied to the arsenic treatment of Surra. The results have been most successful. The recovered animals are being kept under further observation in the Transport Corps.

Pony C 48. 550 lbs.

1st day 600 c.c. ars. ac. sol.	8th day 800 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.	9th ,, 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.	10th ,, 2 gms. ars. ac.
Four days interval.	Four days interval.

15th day 1000 c.c. ars. ac. sol.
16th ,, 75 c.c. atoxyl.
17th ,, 2 gms. ars. ac.
Eight days interval.
26th day 1000 c.c. ars. ac. sol.
27th ,, 60 c.c. atoxyl.
28th ,, 1·5 gms. ars. ac.

No return of trypanosomes. Kept under observation 3 months.

Pony C 49. 580 lbs.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
Four days interval.
8th day 800 c.c. ars. ac. sol.
9th ,, 60 c.c. atoxyl.
10th ,, 2 gms. ars. ac.

This pony showed symptoms of poisoning after the last dose. No further treatment was given.

No return of trypanosomes. Kept under observation 3 months.

Pony C 50. 540 lbs.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
Four days interval.
8th day 800 c.c. ars. ac. sol.
9th ,, 60 c.c. atoxyl.
10th ,, 2 gms. ars. ac.
Four days interval.
15th day 1000 c.c. ars. ac. sol.
16th ,, 75 c.c. atoxyl.
17th ,, 2 gms. ars. ac.
Eight days interval.
26th day 800 c.c. ars. ac. sol.
27th ,, 60 c.c. atoxyl.
28th ,, 2 gms. ars. ac.

No return of trypanosomes. Kept under observation 3 months.

Pony C 51. 650 lbs.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
Four days interval.
8th day 800 c.c. ars. ac. sol.
9th ,, 60 c.c. atoxyl.
10th day 2 gms. ars. ac.
Four days interval.
15th day 1000 c.c. ars. ac. sol.
16th ,, 75 c.c. atoxyl.
17th ,, 2·5 gms. ars. ac.

No further treatment was given as the pony showed symptoms of poisoning after last dose.

No return of trypanosomes. Kept under observation 3 months.

Pony C 52. 520 lbs.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
Four days interval.
8th day 800 c.c. ars. ac. sol.
9th ,, 60 c.c. atoxyl.
10th ,, 2 gms. ars. ac.
Four days interval.
15th day 1000 c.c. ars. ac. sol.
16th ,, 75 c.c. atoxyl.
17th ,, Dose not given as animal had colic.
Eight days interval.
26th day 1000 c.c. ars. ac. sol.
27th ,, 60 c.c. atoxyl.
28th ,, 2 gms. ars. ac.

No return of trypanosomes. Kept under observation 3 months.

Pony C 53. 580 lbs.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
Four days interval.
8th day 800 c.c. ars. ac. sol.
9th ,, 60 c.c. atoxyl.
10th ,, 2 gms. ars. ac.
Four days interval.
15th day 1000 c.c. ars. ac. sol.
16th ,, 75 c.c. atoxyl.
17th ,, 2·5 gms. ars. ac.
Eight days interval.
26th day 1000 c.c. ars. ac. sol.
27th ,, 75 c.c. atoxyl.
28th ,, 2 gms. ars. ac.

No return of trypanosomes. Kept under observation 3 months.

Pony C 54. 570 lbs.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
Four days interval.
8th day 800 c.c. ars. ac. sol.
9th ,, 60 c.c. atoxyl.
10th day 2 gms. ars. ac.
Eight days interval.
19th day 1000 c.c. ars. ac. sol.
20th ,, 75 c.c. atoxyl.
21st ,, 2·5 gms. ars. ac.

No return of trypanosomes. Kept under observation 3 months.

Pony C 55. 600 lbs.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
Four days interval.
8th day 800 c.c. ars. ac. sol.
9th ,, 60 c.c. atoxyl.
10th ,, 2 gms. ars. ac.
Four days interval.
15th day 1000 c.c. ars. ac. sol.
16th ,, 75 c.c. atoxyl.
17th ,, 2·5 gms. ars. ac.
Eight days interval.
26th day 1000 c.c. ars. ac. sol.
27th ,, 75 c.c. atoxyl.
28th ,, 2·5 gms. ars. ac.

No return of trypanosomes. Kept under observation 3 months.

Pony C 56. 660 lbs.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
Four days interval.
8th day 800 c.c. ars. ac. sol.
9th ,, 60 c.c. atoxyl.
10th ,, 2 gms. ars. ac.
Four days interval.
15th day 1000 c.c. ars. ac. sol.
16th ,, 75 c.c. atoxyl.
17th ,, 2·5 gms. ars. ac.
Eight days interval.
26th day 1000 c.c. ars. ac. sol.
27th ,, 60 c.c. atoxyl.
28th ,, 2 gms. ars. ac.

No return of trypanosomes. Kept under observation 3 months.

Pony C 57. 550 lbs.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
Four days interval.
8th day 800 c.c. ars. ac. sol.
9th ,, 60 c.c. atoxyl.
10th ,, 2 gms. ars. ac.

No further treatment was given as the animal showed symptoms of poisoning after last dose and remained dull and off feed for several days.

No return of trypanosomes. Kept under observation 3 months.

Pony C 58. 460 lbs.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
Four days interval.
8th day 800 c.c. ars. ac. sol.
9th ,, 60 c.c. atoxyl.
10th ,, 2 gms. ars. ac.

The pony showed symptoms of poisoning and was dull and off feed for several days. Treatment was suspended. Trypanosomes reappeared in the circulation 23 days after last dose. The animal was then put on treatment with atoxyl and arsenic in alternate doses.

1st day 60 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
5th ,, 60 c.c. atoxyl.
7th ,, 1·5 gms. ars. ac.
9th day 60 c.c. atoxyl.
11th ,, 2 gms. ars. ac.
13th ,, 60 c.c. atoxyl.
15th ,, 2 gms. ars. ac.

No return of trypanosomes. Kept under observation 3 months.

Pony C 59. 540 lbs.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
Four days interval.
8th day 800 c.c. ars. ac. sol.
9th ,, 60 c.c. atoxyl.
10th ,, 2 gms. ars. ac.

The pony showed symptoms of poisoning and was dull and off feed for four days. Treatment was suspended for 25 days. No relapse occurred, but as there was a doubt whether a cure had been effected the animal was put on a course of atoxyl and arsenic in alternate doses.

1st day 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
5th ,, 60 c.c. atoxyl.
7th ,, 1·5 gms. ars. ac.
9th ,, 60 c.c. atoxyl.
11th day 2 gms. ars. ac.
13th ,, 60 c.c. atoxyl.
15th ,, 2 gms. ars. ac.
17th ,, 60 c.c. atoxyl.
19th ,, 2 gms. ars. ac.

No return of trypanosomes. Kept under observation 3 months.

Pony C 60. 650 lbs.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
Four days interval.
8th day 800 c.c. ars. ac. sol.
9th ,, 60 c.c. atoxyl.
10th ,, 2 gms. ars. ac.

A relapse occurred three days after last dose. The animal was then treated with atoxyl and arsenic in alternate doses.

1st day 60 c.c. atoxyl.
3rd ,, 2 gms. ars. ac.
5th ,, 60 c.c. atoxyl.
7th ,, 2 gms. ars. ac.
9th ,, 60 c.c. atoxyl.
11th day 2 gms. ars. ac.
13th ,, 60 c.c. atoxyl.
15th ,, 2 gms. ars. ac.
17th ,, 60 c.c. atoxyl.
19th ,, 2 gms. ars. ac.

No return of trypanosomes. Kept under observation 3 months.

Pony C 61. 600 lbs.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
Four days interval.
8th day 800 c.c. ars. ac. sol.
9th ,, 60 c.c. atoxyl.
10th ,, 2 gms. ars. ac.
Four days interval.
15th day 1000 c.c. ars. ac. sol.
16th ,, 75 c.c. atoxyl.
17th ,, 2 gms. ars. ac.
Eight days interval.
26th day 1000 c.c. ars. ac. sol.
27th ,, 60 c.c. atoxyl.
28th ,, 2 gms. ars. ac.

Trypanosomes reappeared 12 days after last dose. The animal was then put on treatment with atoxyl and arsenic in alternate doses.

1st day 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
5th ,, 60 c.c. atoxyl.
7th ,, 1·5 gms. ars. ac.
9th ,, 60 c.c. atoxyl.
11th day 2 gms. ars. ac.
13th ,, 60 c.c. atoxyl.
15th ,, 2 gms. ars. ac.
17th ,, 60 c.c. atoxyl.
19th ,, 2 gms. ars. ac.

No return of trypanosomes. Kept under observation 3 months.

Pony C 62. 580 lbs.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.

The pony showed symptoms of poisoning after last dose. Treatment was suspended for three weeks. A relapse occurred, and the animal was then treated with atoxyl and arsenic in alternate doses.

1st day 60 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
5th ,, 60 c.c. atoxyl.
7th ,, 1·5 gms. ars. ac.
9th ,, 60 c.c. atoxyl.
11th day 2 gms. ars. ac.
13th ,, 60 c.c. atoxyl.
15th ,, 2 gms. ars. ac.
17th ,, 60 c.c. atoxyl.
19th ,, 2 gms. ars. ac.

No return of trypanosomes. Kept under observation 3 months.

Pony C 63. 500 lbs.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
Four days interval.
8th day 700 c.c. ars. ac. sol.
9th ,, 60 c.c. atoxyl.

The pony showed symptoms of poisoning. Treatment was suspended for three weeks. No relapse occurred in that time. Treatment was then changed to atoxyl and arsenic in alternate doses.

1st day 60 c.c. atoxyl.
3rd ,, 1 gm. ars. ac.
5th ,, 60 c.c. atoxyl.
7th ,, 1·5 gms. ars. ac.
9th ,, 60 c.c. atoxyl.
11th day 1·5 gms. ars. ac.
13th ,, 60 c.c. atoxyl.
15th ,, 1·5 gms. ars. ac.
17th ,, 60 c.c. atoxyl.
19th ,, 2 gms. ars. ac.

No return of trypanosomes. Kept under observation 3 months.

Pony C 64. 580 lbs.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
Four days interval.
8th day 800 c.c. ars. ac. sol.
9th ,, 60 c.c. atoxyl.
10th ,, 2 gms. ars. ac.
Four days interval.
15th day 1000 c.c. ars. ac. sol.
16th ,, 75 c.c. atoxyl.
17th ,, 2·5 gms. ars. ac.
Eight days interval.
26th day 1000 c.c. ars. ac. sol.

The pony had colic after last dose and no further treatment was given.

No return of trypanosomes. Kept under observation 3 months.

Pony B 6. 580 lbs.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
Four days interval.
8th day 800 c.c. ars. ac. sol.
9th ,, 60 c.c. atoxyl.
10th ,, 2 gms. ars. ac.
Four days interval.
15th day 1000 c.c. ars. ac. sol.
16th ,, 60 c.c. atoxyl.
17th ,, 2 gms. ars. ac.
Eight days interval.
26th day 1000 c.c. ars. ac. sol.
27th ,, 60 c.c. atoxyl.
28th ,, 1·5 gms. ars. ac.

This pony died 23 days after completion of treatment from injuries received by being cast in the stall at night. No relapse had occurred.

Pony B 7. 540 lbs.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
Four days interval.
8th day 800 c.c. ars. ac. sol.
9th ,, 60 c.c. atoxyl.
10th ,, 2 gms. ars. ac.
Four days interval.
15th day 1000 c.c. ars. ac. sol.
16th ,, 75 c.c. atoxyl.
17th ,, 2·5 gms. ars. ac.
Eight days interval.
26th day 800 c.c. ars. ac. sol.

On the 26th day, about two hours after the dose of ars. ac. solution had been given, the pony had a severe attack of tympanitic colic and died in a few hours.

Pony B 8. 550 lbs.

1st day 600 c.c. ars. ac. sol.
2nd ,, 60 c.c. atoxyl.
3rd ,, 1·5 gms. ars. ac.
Four days interval.
8th day 800 c.c. ars. ac. sol.
9th day 60 c.c. atoxyl.
10th ,, 2 gms. ars. ac.
Four days interval.
15th day 800 c.c. ars. ac. sol.

About two hours after the dose of ars. ac. solution the pony had a severe attack of tympanitic colic and died in a few hours.

The cause of these two fatalities is not known. The dose of arsenic was not sufficient to cause poisoning, nor did the symptoms or post mortem appearances support this view. The arsenic solution had been made in a large quantity 10,000 c.c. It was several days old. Five other ponies which received relatively small doses of the same brew of arsenic solution showed similar symptoms of tympanitis but recovered on being treated by hypodermic injections of strychnia. The two cases which succumbed received the usual treatment with stimulants and carminatives which was of no avail.

Rules for treatment of Surra.

The following instructions were issued in September 1909 as a guide to veterinary officers in India who proposed to apply the arsenic treatment to Surra cases in their district. As it was pointed out that the preparations and transport of arsenic solutions would be a difficulty in district work, the method of treatment with atoxyl and arsenic in bolus was advised.

Rules for Surra Treatment.

I. Solutions of Atoxyl should be freshly prepared before use in distilled or boiled water. The water should be allowed to cool before the solution is made. Carbolic Acid should not be used for sterilizing as it decomposes the Atoxyl. The solution is to be injected subcutaneously with the usual aseptic precautions.

The Atoxyl by itself has no curative effect, but exercises a rapid action in clearing the circulation of mature trypanosomes. It is, therefore, used only when trypanosomes are present in the circulation. The use is indicated at the commencement of treatment. If the first dose does not result in the complete disappearance of trypanosomes from the blood on the following day, a second injection of Atoxyl should be given 24 hours after the first dose. Atoxyl should be kept in the dark. The Atoxyl and Arsenic should be given after feeding.

II. The injection of Atoxyl has to be followed by ten doses of Arsenious Acid in ball. Care must be taken in making up the balls and in mixing the drug with other ingredients.

The doses which have been determined for horses according to their weight are detailed in the three attached tables.

An interval of one day is allowed between each dose.

The amount of Arsenious Acid is gradually increased. If after any dose the animal is dull or off feed, the next dose must be suspended till the symptoms have passed off. If after any doses the animal is off feed and uneasy and showing symptoms of colic, this shows that the animal cannot tolerate the amount of Arsenious Acid in that dose, and treatment must be suspended till the animal has recovered and the next lower dose used and no further increase in the following doses to be made. Animals showing symptoms of colic are to be treated with opium, chlorodyne or other sedative.

Should trypanosomes reappear in the blood during treatment with Arsenic, Atoxyl should again be used to clear the circulation of the parasites after which the Arsenic is to be continued.

III. Daily examination of the blood should be made and the result recorded with the daily temperature in the observation charts. After the completion of the treatment the animal should be kept under observation, the blood being examined daily (or at least twice a week) for a period of two months.

If a relapse occurs after the first course of treatment a second similar course should be given with slightly increased doses of Arsenic.

IV. Throughout the treatment the animal should receive a liberal diet and walking exercise.

This treatment in our hands has given 75 per cent. of recoveries.

V. If the animal is in a very advanced stage of Surra and in weak condition, it should be treated with Atoxyl injection alone at intervals of 3 to 4 days and receive care and full diet until it has sufficiently recovered to stand the doses of Arsenic.

Cobs.

Body weight from 300 to 500 lbs.

1st day	...	50 c.c. of 4 per cent. Atoxyl subcutaneously.
3rd ,,	...	1·0 gramme of Arsenious Acid in ball.
5th ,,	...	1·0 ,, ,, ,,
7th ,,	...	1·25 ,, ,, ,,
9th ,,	...	1·25 ,, ,, ,,
11th ,,	...	1·50 ,, ,, ,,
13th ,,	...	1·50 ,, ,, ,,
15th ,,	...	1·75 ,, ,, ,,
17th ,,	...	1·75 ,, ,, ,,
19th ,,	...	2·00 ,, ,, ,,
21st ,,	...	2·00 ,, ,, ,,

Light Cavalry Horses.

Body weight from 500 to 800 lbs.

1st day	...	75 c.c. of 4 per cent. Atoxyl subcutaneously.
3rd ,,	...	1·0 gramme of Arsenious Acid in ball.
5th ,,	...	1·0 ,, ,, ,,
7th ,,	...	1·5 ,, ,, ,,
9th ,,	...	1·5 ,, ,, ,,
11th ,,	...	1·75 ,, ,, ,,
13th ,,	...	1·75 ,, ,, ,,
15th ,,	...	2·00 ,, ,, ,,
17th ,,	...	2·00 ,, ,, ,,
19th ,,	...	2·5 ,, ,, ,,
21st ,,	...	2·5 ,, ,, ,,

Heavy Cavalry and Artillery Horses.

Body weight from 800 to 1000 lbs., and upwards.

1st day	...	100 c.c. of 4 per cent. Atoxyl subcutaneously.
3rd ,,	...	1·0 gramme of Arsenious Acid in ball.
5th ,,	...	1·0 ,, ,, ,,
7th ,,	...	1·5 ,, ,, ,,
9th ,,	...	1·5 ,, ,, ,,
11th ,,	...	2·0 ,, ,, ,,
13th ,,	...	2·0 ,, ,, ,,
15th ,,	...	2·5 ,, ,, ,,
17th ,,	...	2·5 ,, ,, ,,
19th ,,	...	3·0 ,, ,, ,,
21st ,,	...	3·0 ,, ,, ,,

Remarks. Large mules will stand the treatment advised for heavy horses, and small mules the treatment for light horses, with the exception that a smaller dose of Atoxyl must be given.

For large mules 60 to 75 c.c. of a 4 per cent., and

For small mules 50 to 60 c.c. of a 4 per cent.

No reports have been received by me on the results of the treatment of Surra in the districts. Captain Dawson in the *Indian Veterinary Journal*, Vol. I. No. 3, states that Mr Gibson, Director of the Vaccine Institute, Madras, has tried the treatment with complete success.

Conclusions.

I. The results herein recorded are further evidence that arsenic is a specific for Surra in horses; that a permanent cure is effected, and not merely a temporary tolerance of the disease; that the treatment is simple, and that by careful dosage 70% and upwards of Surra cases, even when contracted spontaneously and in the last stage, can be cured.

II. Arsenic is best administered in form of arsenious acid, in bolus or in solution. Atoxyl is a convenient form of giving arsenic hypodermically.

The methods of dosage which have been found successful are:

(1) Arsenious acid alone. This is given in bolus, in gradually increased doses, with one day's interval between each dose. Eight to ten doses are sufficient.

(2) Atoxyl and arsenious acid, given alternately in gradually increased doses, with one day's interval between each dose. Five doses of atoxyl and five doses of arsenious acid are sufficient.

(3) Arsenious acid in solution, atoxyl and arsenious acid in bolus. The dose of arsenious acid solution is followed by atoxyl, and arsenious acid, in bolus, on successive days. The doses are repeated once or twice after an interval of four days between each series, and finally after an interval of eight days.

III. The success of the arsenic treatment of Surra depends chiefly on the strict observance of the following principles:

(*a*) Arsenic must be given in full sub-toxic doses. Care should be taken, especially in the earlier part of the treatment, not to administer doses sufficiently large to cause symptoms of poisoning, such as colic, loss of appetite etc. If this occurs, treatment should be suspended till the animal has completely recovered. In the meantime, occasional injections of atoxyl should be given as required.

(*b*) Arsenic must be administered at intervals, and not continuously day by day. In the systems of dosage (1) arsenious acid alone, and (2) atoxyl and arsenious acid alternately, experience has proved that one or two days' interval between doses is necessary. In many experiments where the doses were given daily, and also when the

interval was extended to three and four days, relapses occurred. In the third system of treatment (arsenious acid solution, atoxyl and arsenious acid in bolus), the interval between the first, second, and third, series of doses should be four days, and eight days between the third and fourth series.

(*c*) If the animal is in a very debilitated condition, it is advisable to clear the circulation of trypanosomes by one or more injections of atoxyl and feed carefully for several days before administering arsenic by mouth.

(*d*) Cases which relapse after treatment should be put on a second course with increased doses, either by the same or a different system of dosage.

In cases of relapse, and also where treatment is not well tolerated, it is often advantageous to change the system of dosage.

(*e*) Whenever possible, cases should be kept under observation for a period of two to three months after completion of treatment. Relapses generally occur within a period of six weeks.

(*f*) It is possible to cure Surra by a single dose of arsenic. This is not a practical method, as the amount of arsenic requisite produces severe symptoms of poisoning and a large percentage of cases ends fatally.

Pony C 7 recovered after a single dose of 1 gm. arsenic in bolus.

Mule C 44 recovered after a single dose of 3 gms. arsenic in bolus.

Pony C 30 recovered after a single subcutaneous injection of 200 c.c. of a 4 % solution of arsacetin.

Pony C 31 recovered after a single subcutaneous injection of 100 c.c. of a 10 % solution of soamin.

PLATES XXII TO XXVII.

Photographs of the Transport Ponies which recovered after treatment for Surra. The first photographs were taken at the time of their arrival at the Laboratory. The second photographs were taken after the three months period of observation before the animals were returned to the Transport Corps.

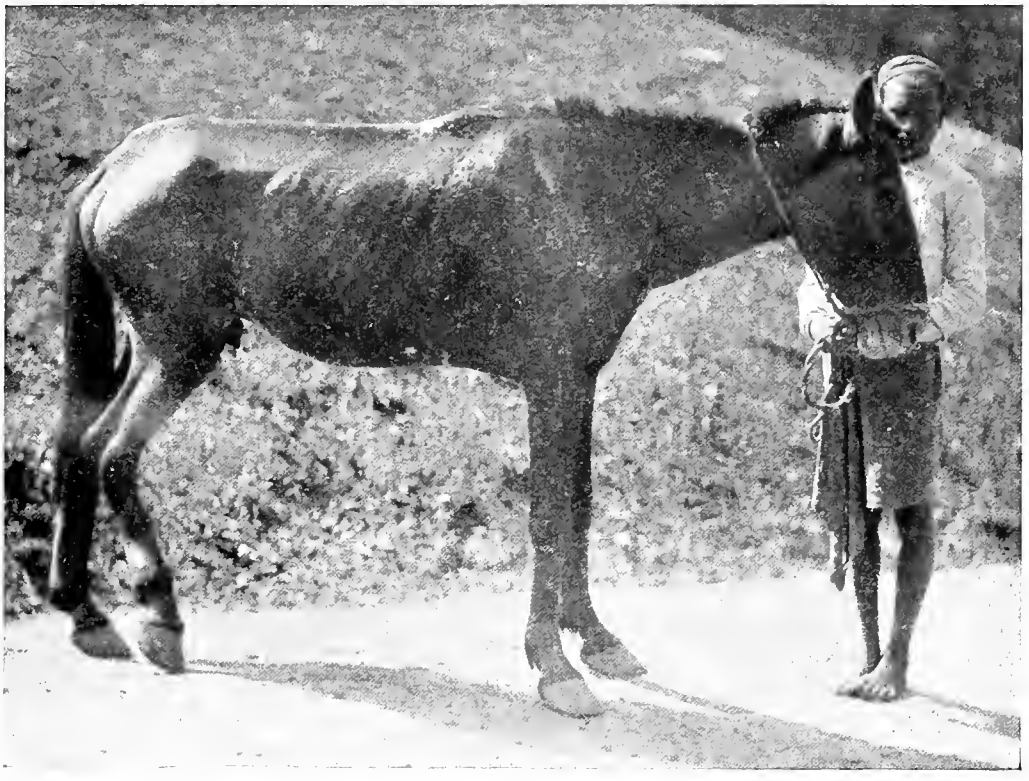

Fig. 1. Pony C 49 before treatment.

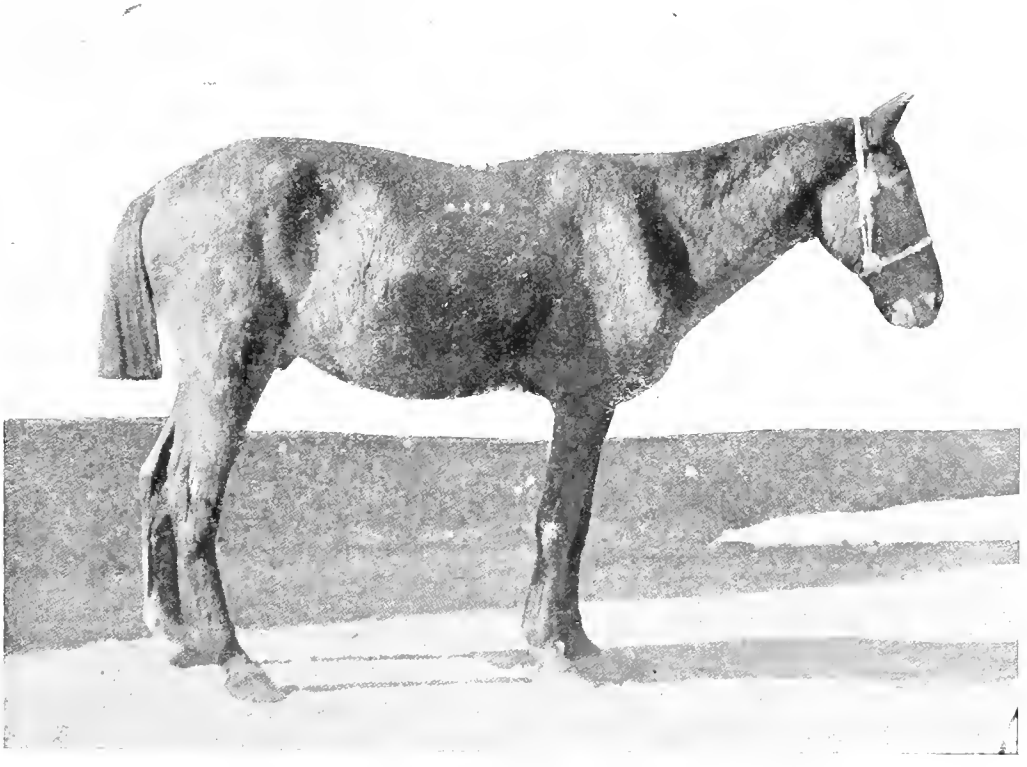

Fig. 2. Pony C 49 after treatment.

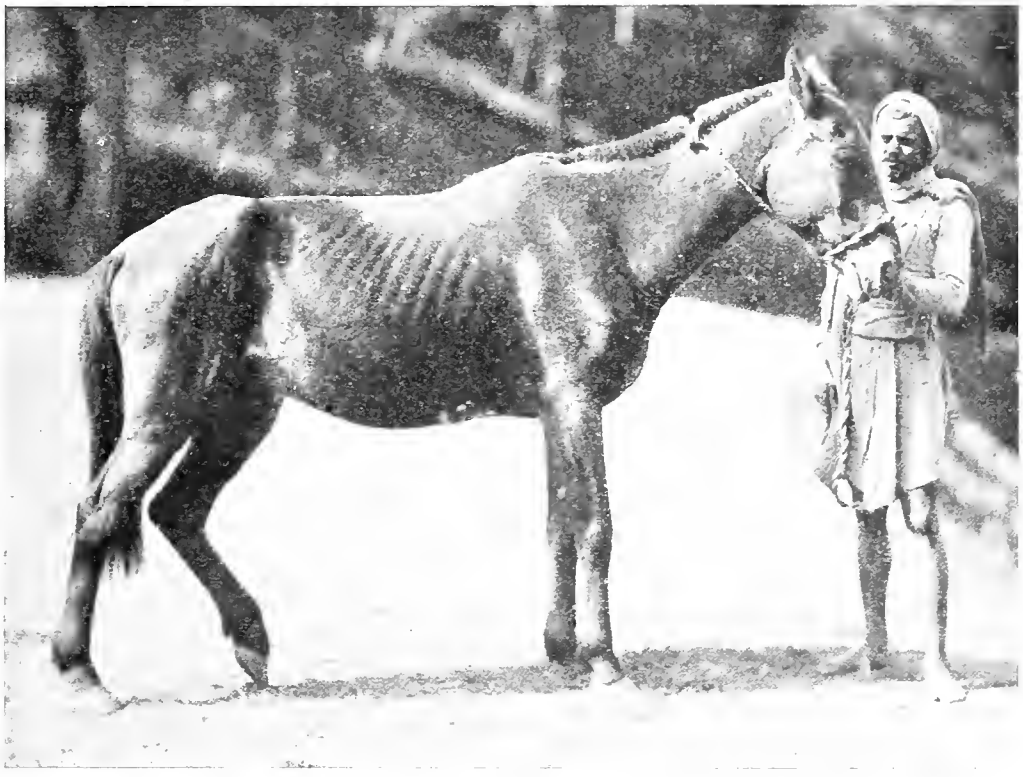

Fig. 1. Pony C 56 before treatment.

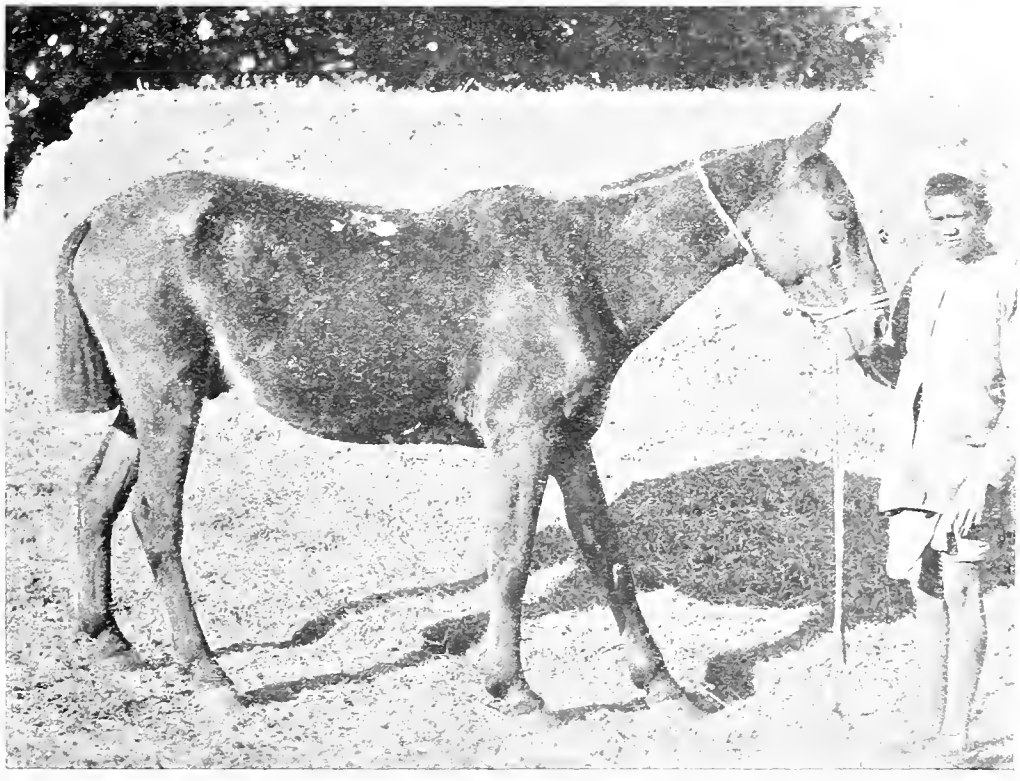

Fig. 2. Pony C 56 after treatment.

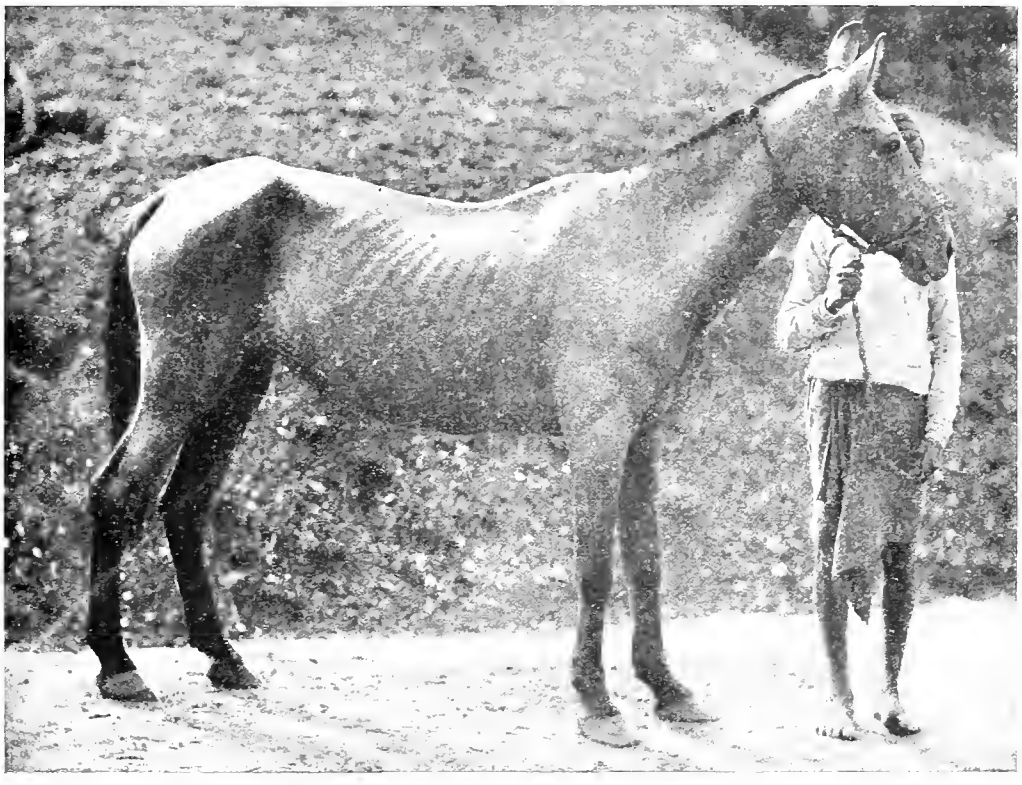

Fig. 1. Pony C 57 before treatment.

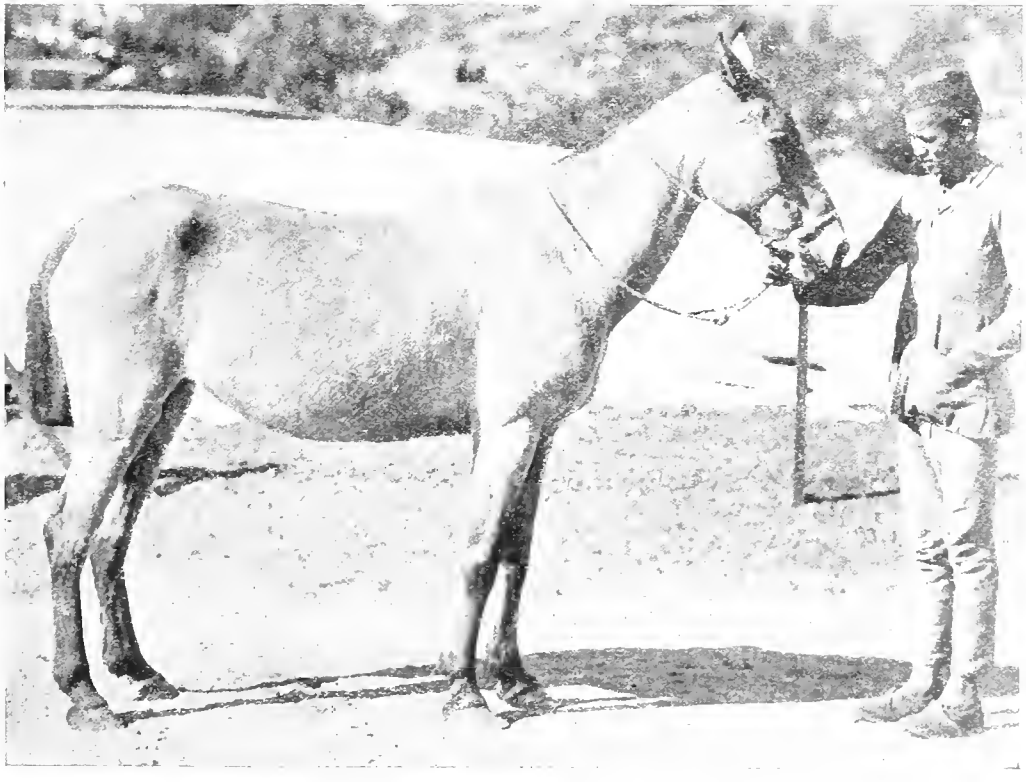

Fig. 2. Pony C 57 after treatment.

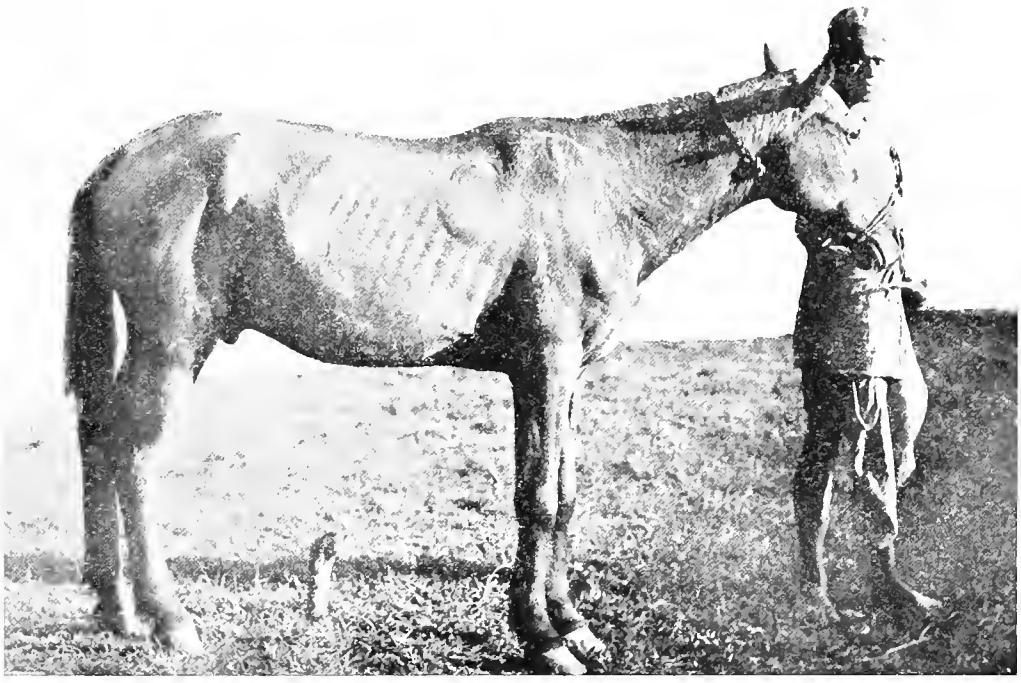

Fig. 1. Pony C 59 before treatment.

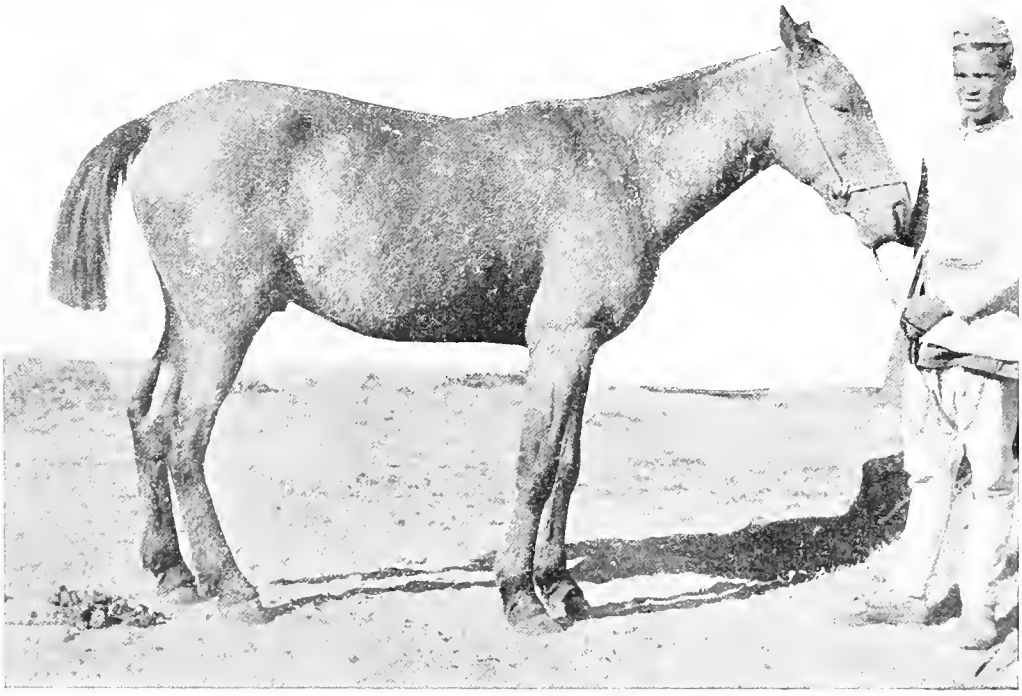

Fig. 2. Pony C 59 after treatment.


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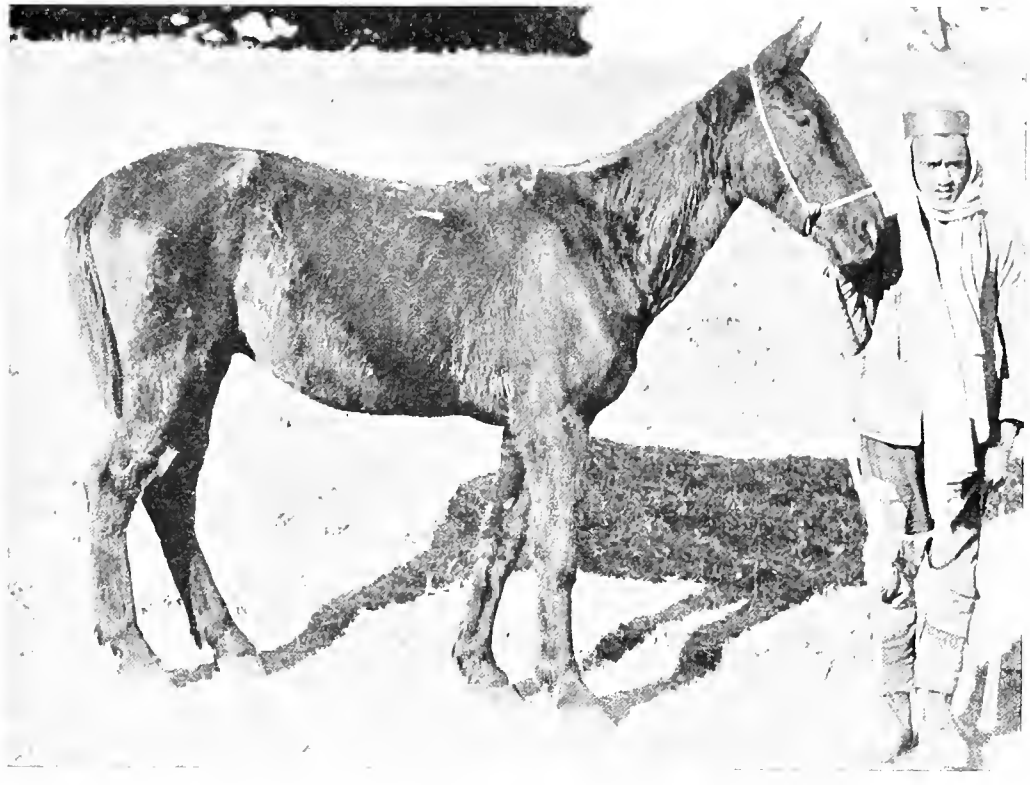


Fig. 1. Pony C 63 before treatment.

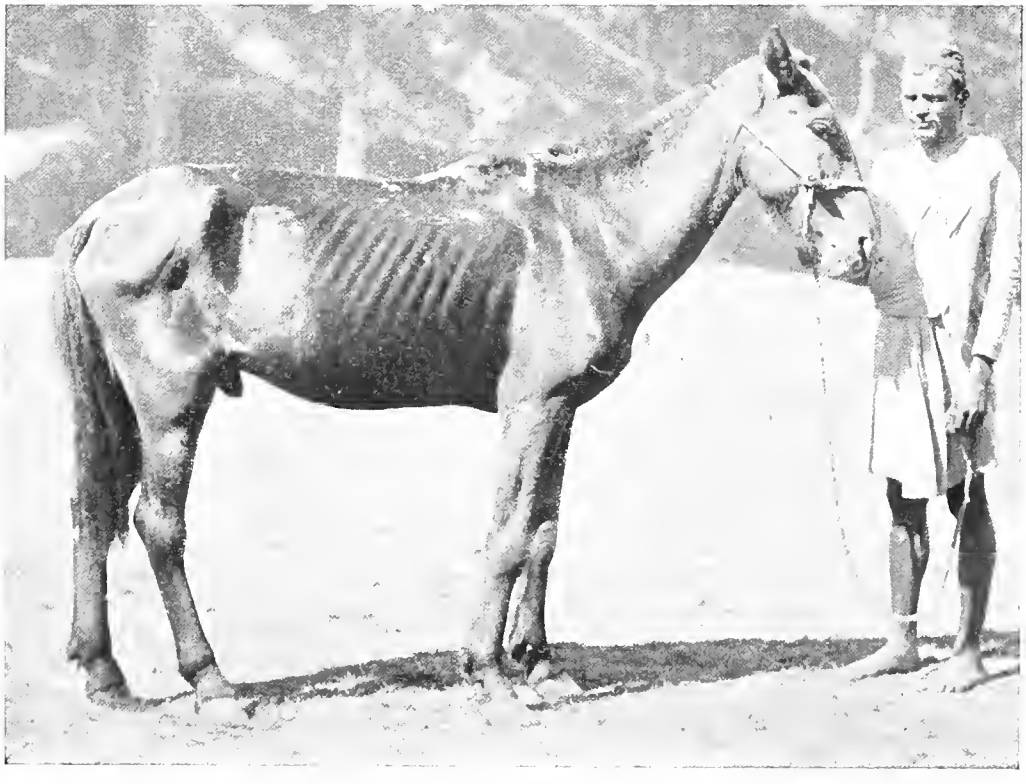


Fig. 2. Pony C 63 after treatment.

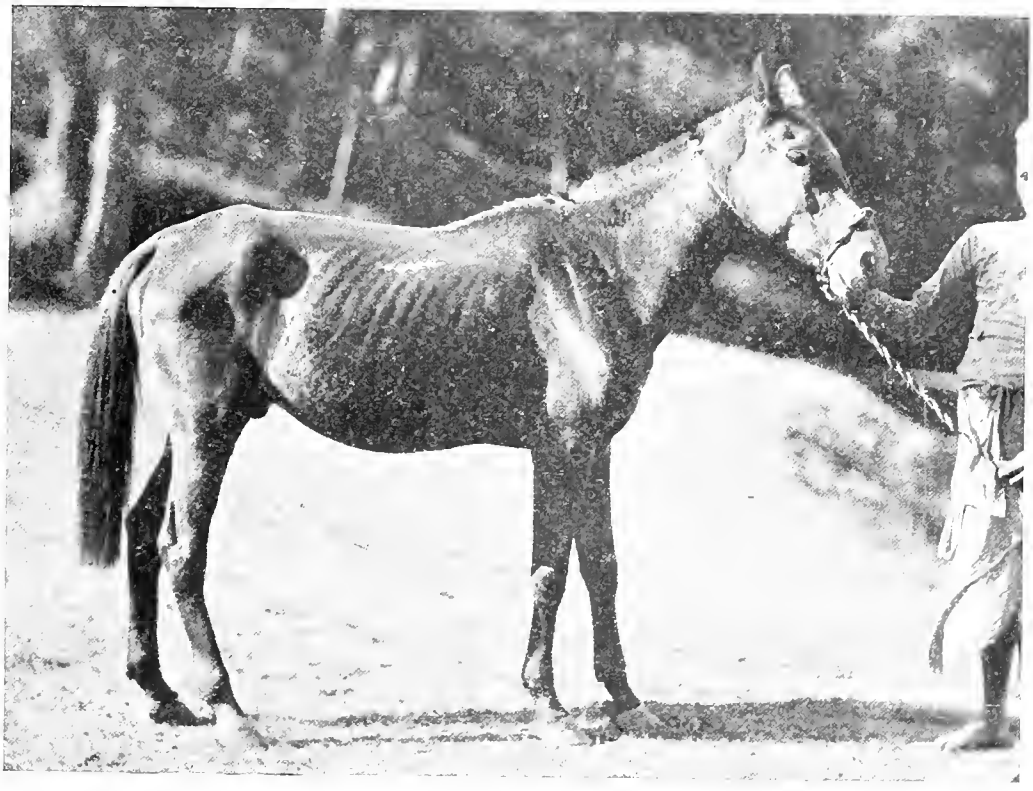


Fig. 1. Pony C 64 before treatment.

Fig. 2. Pony C 64 after treatment.

PIROPLASMOSIS AMONG CATTLE IN THE MOMBERA DISTRICT, NYASALAND, 1909.

By HUGH S. STANNUS, M.B. London.
Medical Officer, Nyasaland.

(With Plate XXVIII.)

During the rainy season of 1908–9 an outbreak of disease among cattle occurred over a somewhat widespread area of the Mombera district, causing considerable losses in herds owned by natives.

The district in question occupies a plateau lying at an elevation of from 3000 to 4000 feet, and inhabited by tribes owning a very large number of cattle. Some years ago they were very much reduced in numbers by an epidemic of Rinderpest, but they have since then rapidly increased and at the present time are estimated at about 22,000 head.

From information elicited from missionaries long resident in the district the disease would appear to have been observed periodically each year during the rains for many years past, occurring with varying incidence and mortality.

I arrived in the district at the end of the rains, April 1909, and was only able to see a few recovering and chronic cases at that time. A small recrudescence of the sickness however occurred at and around Loudon during the months of October and November of the same year, and from these cases I was able to arrive at certain facts elucidating the nature of the disease.

Epidemicity. The disease recurs annually during the rains, namely November to April, and occasionally cases may be met with as late as June. The incidence and mortality vary considerably in different years, in different parts of the district, and among herds in adjoining

villages under exactly the same conditions. Information obtained from natives is of course unreliable, but judging from data collected in villages where the sickness had been most prevalent, it was gathered that the mortality would appear to have been about 10 per cent., and occurred among adult animals.

Symptoms. The disease is usually of an acute nature, the animal being rapidly reduced so that death may occur in three or four days after the first appearance of symptoms. Others again recover after a few days' illness, and at the end of a week appear normal. In some a chronic condition supervenes, the animal lingering on for months, ultimately recovering, or in other cases dying. The coat is staring, the hump limp, and the head hanging down; running from the eyes and nostrils is commonly seen. The beast continues to feed to within 24 hours of death; its gait becomes more unsteady as the disease progresses, and later it drops to the ground unable to rise again. Respiration is rapid and accompanied by a certain amount of dyspnoea. The bowels are often constipated, but diarrhoea may supervene; occasionally mucus and blood have been observed in the stools. The urine may be of a red colour, but "redwater" has not been noticed by natives.

Post-Mortem Examination reveals conditions differing only in degree, characterised by a haemorrhagic oedematous process and enlargement of the gall-bladder. A varying amount of fluid is found in the serous cavities; small subpleural, subpericardial and subperitoneal haemorrhages, about 1—10 mm. in diameter, are found. The lungs and heart show no macroscopical changes. The liver presented no haemorrhages under its peritoneal covering, its substance appeared normal (some mottling in one case), and no haemorrhages nor infarcts were observed. The gall-bladder and its duct together with the bile passages were all dilated, the former in marked degree, with haemorrhage and oedema in its walls; denudation of epithelium had occurred and it contained much mucus; the bile passages were much dilated and sacculated with fibroid and calcareous changes in the walls. In these and in the gall-bladder numerous flukes were present. The kidneys and ureters were surrounded by a haemorrhagic oedema, more or less marked. On section, the kidneys showed minute subcapsular haemorrhages with haemorrhagic oedema in the neighbourhood of the hilum; in some cases no changes were present in or around the kidneys. There were no definite infarcts. The urinary bladder appeared normal but contained urine of a reddish tinge suggesting the presence of blood

pigment, but no means of testing for this were available. The suprarenal capsules were of a deep yellow colour. In one case the right kidney contained sixteen small calculi, the largest being of the size of a pea. The spleen appeared normal to the unaided eye, in consistence, colour, etc. Subperitoneal haemorrhages were marked in the omentum and mesentery. The fourth stomach showed submucous haemorrhages in one case. In the large and small intestines there were occasional haemorrhages into the substance of their walls. The superficial lymphatic glands showed no marked enlargement; those at the base of the tongue also were not enlarged. Those in the posterior mediastinum, at hilum of liver, in mesentery, etc., were enlarged, of a grayish colour, diffluent and contained a large amount of fluid. The fat in one case was of a deep yellow colour as if bile-stained.

Microscopical Examination was confined to the blood during life, and to smears from various organs and blood after death.

Sick animals, in which a fatal termination had occurred, invariably showed organisms in their blood: they were never found in any very large numbers and did not apparently increase in number rapidly as the illness progressed. In those animals recovering rapidly after a few days' illness and examined after a week's interval, I was unable to find parasites in the peripheral blood. On the other hand in a few animals which had been ill for several months and still presented symptoms such as staring coat, emaciation and a certain degree of listlessness, I found organisms in the blood but in small numbers. All examinations were made by means of smears stained with Leishman's stain.

The peripheral blood, during life and subsequent to death, showed a small piroplasm[1] to be present in moderate numbers, rod-shaped and comma-shaped types being commonest; some of the former occurred in pairs, with varying separation noticeable at the red chromatin-stained poles as if division were taking place, with the production in some cases of a rod-shaped body with bipolar red staining. A few small ring-forms were also present but in much smaller numbers, showing a central vacuole with chromatin usually collected at one point. Ovoid forms were rather more common, with as before a central vacuole. Another form showed characters midway between this ovoid form and the bacillary, that is to say with a chromatin mass at one end; the rod

[1] In a drawing accompanying Dr Stannus' paper, parasites are figured which appear identical with *Theileria parva*. The corpuscles contained forms similar to those illustrating the paper by Nuttall and Fantham, *Parasitology*, III. No. 2, Pl. XII, especially figs. 1, 3, 15, 20, 25.—G. H. F. N.

is wider in the middle, with a slight appearance of a vacuole. The size of the rods was from 1 to 2 μ; the rings were about 1 μ in diameter and ovoid forms 1·5 by ·75 μ.

In the chronic cases coccoid and rod-shaped organisms predominated; the number of parasites being very small and in no cases were they numerous.

There was some variation in the size of the erythrocytes and poikilocytosis was noted. In the chronic cases there was a very considerable degree of anaemia.

Smears from the spleen presented a limited number of erythrocytes containing parasites. Smears from liver, kidneys and glands showed no parasites.

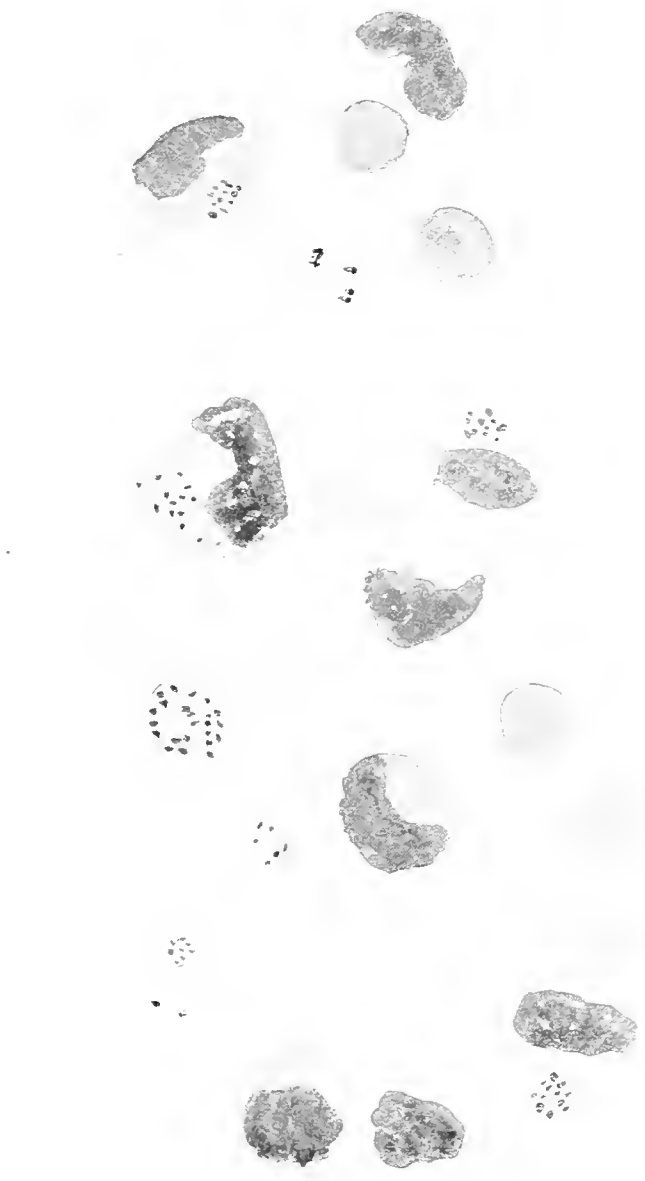
Inoculability. Inoculation experiments were commenced but had to be given up, and no results were obtained.

Remarks. The type of piroplasmosis under consideration does not appear to correspond with East Coast Fever (*P. parvum*), while the microscopical characters of the parasite are not those of *P. mutans*; the *P. bigeminum* type being of course out of the question. It would appear to me that we are dealing with a form (probably one of many to be found in Africa) having affinities with those recently found in Uganda, and I believe in the Sudan.

Since leaving the Mombera District, the disease has continued and many more animals have succumbed; spleen smears from some of these animals have been received and I have had the opportunity of staining them by Giemsa's method. Numerous piroplasmata have been demonstrated in the erythrocytes contained in the spleen and the so-called "*blue bodies*" (Koch's Granules) have been found. (See Plate XXVIII.)

These bodies have been looked upon by various authorities as pathognomonic of East Coast Fever and latterly on the finding of these elements a diagnosis has been made, while symptoms and post-mortem findings have been relegated to a secondary place. If this is to hold good then the disease in question in this country is East Coast Fever, and in this light it is interesting to compare it with one recently described by Col. Sir David Bruce and his colleagues in Uganda under the native name "Amakebe" (*Proceed. Roy. Soc. B.* Vol. 82, 1910), a disease very fatal to calves there and which he concludes is East Coast Fever.

Interest lies in the fact that "blue bodies" were found in the spleen, etc. (rarely in the blood) though some of the universally described pathological lesions such as infarcts in the kidneys were



Illustrating the paper by Dr H. S. Stannus on Piroplasmosis in Nyasaland cattle. From a coloured drawing by the author representing appearances observed in spleen smears: Red blood corpuscles, leucocytes with and without inclusions: bodies taking the chromatin stain. Breaking down leucocytes, leucocyte nuclei, and "blue bodies."

wanting in many cases. It is conceivable that considerable variation may occur in the lesions, due to variable intensity of the infection, etc., and this might explain the absence of infarcts, of enlarged glands in the infrascapular regions in the cases observed by myself, in which the jelly-like collections were replaced by what I have described as a haemorrhagic-oedematous process.

One of the chief points of difference however lies in the fact that "amakebe" attacks young animals, the reverse being more commonly seen in this country, and again here the occurrence of apparently chronic cases has been noticed.

I have not seen any suggestions as to the nature of the "blue bodies" but in various spleen smears seen by myself, appearances would seem to show conclusively that they have origin in the breaking up of large mononuclear leucocytes. (Plate XXVIII.) Smears show these cells as in normal spleen smears but also others with red granules in their blue protoplasm; various stages may be seen of the breaking up of these cells with the separation of the cytoplasm containing the red granules and the assumption of the latter of a more or less circular form; in many cases the bare nucleus is seen with the rounded blue body lying at its side. Thus I am of opinion that the "blue body" is a part of a large mononuclear leucocyte; whether the red granules within them are of a protozoal nature is undetermined.

PLATE XXVIII.

Koch's "blue bodies" encountered in spleen smears of Nyasaland cattle. (See concluding paragraph of the text and legend at foot of Plate.)

NOTE ON ANKYLOSTOMIASIS IN NATAL.

By ERNEST HILL, M.R.C.S. Engl., L.R.C.P. London,
D.P.H. Cambridge.
Health Officer for the Colony of Natal.

At the present time *Ankylostoma* infection in man and disability resulting therefrom has aroused much concern. The geographical origin and present distribution of the two species of *Ankylostoma* known to be parasitic to man are of considerable interest, even though it be only academic. It is, therefore, probable that the following note may be worthy of publication.

At the end of 1909 I received by courtesy of Dr Staunton, a Medical Officer in the service of the Indian Immigration Trust Board of Natal, 824 *Ankylostoma* worms, obtained from the faeces of 18 Indians after treatment. I found 132 of the species *Ankylostoma duodenale,* 51 males and 81 females, and 692 of the species *Uncinaria americana* or *Necator americanus,* 220 males and 472 females. In seven cases the latter only was found to the total 218.

The Indians had been resident in Natal, one for more than 10 years, one for six years, two for three years, and three for one year. In 11 cases both species were found, the duration of residence in Natal being seven years (one), four years (five), three years (three), two years (one), one year (one). The largest number of *Necator* found in any one case was 169 (with one *Ankylostoma duodenale*) and the smallest five (with 54 *A. duodenale*). The largest number of *A. duodenale* was 54, the host having been resident about one year, and the smallest number one.

I have not had opportunity of examining worms from Indians at time of arrival from India but from unpublished official reports of Mr B. A. Nicol, Medical Superintendent to the Indian Immigration Trust Board of Natal, it appears that Indians recruited in Madras Presidency are infested principally with *Necator americanus.*

Table showing species and sex of Ankylostoma worms discharged in faeces of each of 18 Indians.

Duration of Residence in years	*Necator americanus*		*Ankylostoma duodenale*		
	Male	Female	Male	Female	Total
Over 10	19	34	0	0	53
7	27	45	19	19	110
6	5	33	—	—	38
4	9	27	1	1	38
4	1	8	1	—	10
4	16	31	8	8	63
4	2	18	—	6	26
4	84	85	—	1	170
3	1	6	—	—	7
3	3	17	1	7	28
3	9	24	1	—	34
3	2	14	1	—	17
3	—	12	—	—	12
2	12	35	2	2	51
1	2	28	—	—	30
1	7	16	—	—	23
1	19	36	—	—	55
1	2	3	17	37	59
	220	472	51	81	824

NOTE OF A CASE OF INTESTINAL INFECTION IN MAN, WITH THE LARVA OF *HOMALOMYIA CANICULARIS*.

By A. BERTRAM SOLTAU, M.D.,
Plymouth.

The specimen of the larva of *H. canicularis* was obtained from the stool of an adult man, who gave the following history:

He had been in good health and had not been conscious of any intestinal disorder. On the morning of the 28th of May he happened to look down into the pan of the closet after passing a motion and noticed that there was considerable movement taking place in the motion, which was partially formed. This movement was due to the presence of a large number of larvae, several of which crawled up the edge of the pan. He secured one and brought it to me in a bottle, and it was then alive and moving vigorously about the bottle. It maintained its vitality so long as it was in my possession, and until it was despatched to Cambridge for identification.

Since the day on which the larvae were passed no more have been seen nor has the patient, once he recovered from the state of natural alarm at finding he was the host of such unpleasant parasites, suffered in any way from intestinal trouble.

He is a man who has been accustomed to eat largely of salads, etc., and it seems probable that the larvae gained an entrance by means of an imperfectly washed salad.

There are no stables in the immediate vicinity of his house.

NOTE ON A CASE OF INTESTINAL MYIASIS.

By J. R. GARROOD, M.D.

The following case of Intestinal Myiasis occurred in a boy about twelve years of age, in normal health, who brought to me in September 1909 what he took to be "worms" discharged with his faeces. He said he did not feel anything abnormal but saw a mass of the creatures upon and in his freshly-voided faeces. He called his mother, and she stated to me afterwards that the "worms were in a cluster like a swarm of bees," they were crawling about the faecal mass which was loose in character, as had been the case for several days. They varied considerably in size, as did the specimens brought to me. The mass of creatures would have filled one or two tablespoons.

The boy was taking his ordinary diet, his meat was probably beef or pork, and may have been imperfectly cooked, he also had cabbage and other vegetables, but not salads or uncooked vegetables.

The home conditions were not the most cleanly and there would probably be no lack of opportunity for the entry of the parasites either in the egg or larval form.

I took specimens of the larvae to the Cambridge Zoological Laboratory and they were sent to Dr David Sharp, who identified them as the larvae of a species of *Homalomyia*[1].

I could not ascertain that the boy suffered any inconvenience from the presence of the larvae in the bowel, save perhaps a little diarrhoea, but he seemed to be mentally disturbed by their exit! There has been no recurrence[2].

[1] I am very much indebted to Dr Shipley, Prof. Nuttall and Mr Hugh Scott for the help they have given me in the preparation of this note.

[2] That the presence of living animals in the bowel may give rise to serious symptoms is shown by a case I saw recently: a boy aged 5½ years complained of pain in the stomach which gradually extended downwards and after three days he passed a centipede (*Lithobius*) in a constipated motion, this was followed by some diarrhoea.

This history seems to be accurate, for the boy's father brought me the centipede and stated that it was partly imbedded in the faecal mass and was alive and moving. The motion was passed into a clean and empty vessel.

Recorded Cases of Intestinal Myiasis.

The following are some of the instances of intestinal myiasis and allied conditions I have found in the literature at my disposal.

Cattle (1906) records a case of intestinal invasion by larvae of the bot-fly with few symptoms, but the larvae were still being discharged seven months after he first saw the patient.

Hutton (1901) describes various symptoms such as vomiting, constipation with diarrhoca, epigastric pain, colic, and even haematemesis. These symptoms may be promptly relieved by the evacuation of the parasites either by the mouth or anus. As large a quantity as a quart has been said to have been expelled at one time. Sometimes the conditions may become chronic, intervals of quiescence being followed by a return of the symptoms.

Thébault (1901) reports *a case of Typhoid-like illness* in a young woman who habitually ate Camembert cheese full of the mites *Piophila casei*. He fed a dog on fifty of the same mites and the animal became ill, passing blood in the faeces. At autopsy the dog's internal organs were found to be congested and the mucous membrane of the small intestine was the seat of numerous small hacmorrhages. Thébault thinks the illness of intestinal myiasis may be due to bacterial invasion of the wounds in the intestinal mucosa, caused by the sharp processes of the larvae, and also to the absorption of poisonous substances from the larvae themselves.

Drew (1906) states that in the horse the stomach may be so closely set with the larvae of the bot-fly that the mucous membrane is invisible, and yet the animal shows no symptoms.

Habits of these flies and their larvae.

Braun (1906) states that *Homalomyia canicularis* is a common fly in houses and that the larvae have been found in decaying vegetable matter, also in the nests of the humble bee.

According to Hewitt (1909) the larval stage of *H. canicularis* lasts from three to four weeks and the pupal stage from two to three weeks. The larvae feed on waste vegetable and faecal matter.

Mode of entry.

The most obvious mode of entry of the larvae or eggs is with the food, and from the observed habits of these insects, vegetable food in an uncooked, or insufficiently cooked condition is probably the vehicle for the introduction of either the actual larvae or of eggs which are about to be hatched. In most cases no doubt these larvae or eggs would be killed by the digestive juices, but not always, for Hutton (1901) states that dipterous larvae have been immersed in water for days without killing them and have lived for from twelve to seventy-two hours in the stomachs of the frog and guinea-pig—long enough in the case of a human host to pass on to the more favourable locality of the large intestine.

Hewitt (1909) says that the eggs of *H. canicularis* may be deposited on the lips or nostrils of children and passing into the stomach and intestines give rise to intestinal myiasis and diarrhoea.

He also states that the larvae may enter the rectum when the patient uses the old style of privy, being presumably splashed up from below.

Hewitt (1907) states that the anthomyidae lay their eggs in the contents of privies as well as elsewhere.

Error in diagnosis.

The occurrence of the larvae in human faeces under the above conditions may give rise to error but I believe this source to be excluded in my case, both by the history as given by the boy and his mother and by the fact that an earth closet was used which would render the mistake less likely.

Description of larvae from my case.

The larvae as brought to me are brown, oval, flattened creatures surrounded by sharp processes bearing spines or lateral branches. There are six rows of these processes, two on either side and two down the back near the mid-dorsal line, the latter have no lateral branches.

The anterior end of the larva is smaller than the posterior but there is no head, a longitudinal skeletal structure shows through the cuticle of the anterior end as two dark bars and two bristle-like processes project forwards from the front of the first segment.

Between the first and second segments on either side are the anterior spiracles ending in a palmate structure having nine lobes.

The posterior spiracles are two knob-like bodies on the dorsal surface of the hind end of the body and in some specimens the air vessels can be seen on either side joining the anterior and posterior spiracles and throwing off branches amongst the tissues.

Owing to the many sharp spine-like processes the larvae become readily covered with dirt, and as the processes point principally backwards intestinal movements would result in a forward displacement of the larva. This and the possible occurrence of retrograde peristalsis will account for some of the cases where the larvae are vomited as well as discharged by the rectum.

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NOTE REGARDING THE NEW BUFFALO SPIROCHAETE.

By ANDREW BALFOUR, M.D.,

Khartoum.

I HAVE been much interested in Professor Nuttall's paper in the April number of *Parasitology* (this volume, p. 113) and especially in his description of a parasite found by him in blood smears from a buffalo sent to England from British East Africa. He has named this organism *Spirochaeta bovis caffris* though he is evidently somewhat doubtful as to its nature. I write to say that early in 1909 I received from Captain Hadow one blood smear from a Jackson Hartebeeste which he had shot in the Bahr-El-Ghazal and on the body of which he found *G. morsitans* in the act of feeding. In this smear I found organisms which answered very closely to those described and figured by Professor Nuttall. They were somewhat smaller, having an average length of 16·5 μ and a breadth of 0·7 μ but presented the same appearance, stained in the same way and in some instances showed the achromatic transverse bands which he mentions. From its shape I mentally termed one variety the buffalo-horn type and I made drawings of the different forms encountered. On April 18th 1909 I sent the slide to Dr Wenyon at the London School of Tropical Medicine with a note directing his attention to these curious parasites and stating that, to me, they looked more like spirochaetes than anything else but that I was unable to classify them. Unfortunately, though my letter was safely delivered, the box containing the slide was never seen again. Dr Wenyon, however, wrote and told me that he had come across similar forms in the blood of big game which had been shot, and recorded his opinion that the forms in question were not blood parasites at all but were derived from the intestine and had been carried into the exit wound by the bullet or by discharges finding their way along the bullet track. One recognised the possibility of such an

occurrence and the fallacies to which it might give rise and, on meeting Captain Hadow, I asked him if he remembered where the animal had been shot. To the best of his recollection the bullet had passed through the neck severing the gullet and it is quite possible that in the last throes stomach contents might have regurgitated through the wound of exit. The presence of very thin thread-like spirochaete forms in the film and of some bodies which suggested yeasts made me refrain from publishing any account of the case until I had more evidence regarding Dr Wenyon's hypothesis.

Quite recently I received from Captain Cummins, S.M.O. Kordofan, a blood smear from a goat which had died from infectious pleuro-pneumonia. He wrote to say that he had found spirochaete-like bodies in the film and solicited my opinion about them. To my surprise, on examining the specimen, I found my hartebeeste parasites or forms very closely resembling them. I wrote to Captain Cummins mentioning the case of the hartebeeste and the view expressed by Dr Wenyon and asking him if the blood sent could have been contaminated from the stomach or intestines. He replied that there was every possibility that this had occurred as, prior to making the film, he had been examining the goat's intestines. Indeed he thought it very likely that such an accident had taken place and agreed with me in thinking that these parasites were of intestinal origin.

I think with these facts before us we would do well to hesitate to accept *Sp. bovis caffris* as a true blood parasite of the buffalo. Perhaps Professor Nuttall would kindly state if there were any other peculiar forms, such as yeast cells, in his preparations or anything pointing to infection from internal organs, while it might be well to ascertain if there was any chance of the blood sample becoming contaminated before or at the time the blood was taken.

I do not write in any critical spirit but because I know by sad experience that there are many pitfalls in blood work in the open in tropical countries and it would be well to make certain if Professor Nuttall's interesting parasites are really, as he thinks, *haematozoa*, in which case it would appear that the Jackson hartebeeste of the Southern Sudan harbours a similar organism. I am inclined, however, to agree with Dr Wenyon, especially in the light of what was found in the case of the goat and to consider these curious bodies as representatives of the flora of the intestinal tract.

Perhaps if this letter catches Dr Wenyon's eye he will give us the benefit of his experience and aid us at arriving at the truth.

REMARKS ON THE FOREGOING NOTE BY DR ANDREW BALFOUR.

By GEORGE H. F. NUTTALL, F.R.S.

Dr Balfour's note raises the question whether *Spirochaeta bovis caffris* is a blood parasite or an organism derived from the alimentary canal owing to the entry of intestinal contents into the blood or blood stream consequent upon the effects of gunshot wounds. I must confess that this source of error did not occur to me in this case.

On again looking very carefully through the blood-films, I have succeeded in detecting a few small bacilli which indicate that the blood may well have become contaminated either whilst in the circulation or after the films were prepared. Mr W. F. Cooper obtained the blood as it welled from a stab in the heart of a buffalo (No. 1) which he had shot, the bullet having passed a little in front of the heart and broken the animal's leg. He felt "sure that there was practically no chance whatever of organisms from the intestines getting into the blood circulation." On the other hand, it must be noted that the films may have become contaminated after they were taken owing to accelerated manipulations consequent upon another buffalo (No. 2) showing a desire to charge Mr Cooper whilst he was engaged in preparing the films. I may add that the blood of buffalo (No. 2) contained no spirochaetes.

Whether the organism is or is not a haematozoon can only be decided by further observations. I am not aware that such forms have been described as occurring in the intestines of animals; they certainly differ very considerably from the typical spirochaetes which various observers have found in the intestine.

A Correction.

I would take occasion to note that, through an oversight, the magnification $\times 3600$ is given in the legends to Plates X and XI, this being the enlargement used when drawing the parasites. The drawings were reduced by $\frac{1}{6}$ for the purpose of reproduction, consequently the magnification should read $\times 3000$ in the legends.

ON THE ENTOZOA OF FISHES FROM THE FIRTH OF CLYDE.

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(With Plate XXIX.)

CONTENTS.

Introduction.

The material on which the following notes are founded was collected during a short residence at the Marine Biological Laboratory, Millport, in August, 1908. A comprehensive survey of the marine fauna was impossible in so limited a time, but a fair idea of the parasites of some of the common fishes was obtained. I have to thank the Government Grant Committee of the Royal Society for enabling me to undertake this work. It is also necessary to gratefully acknowledge the courtesy of several members of the West of Scotland Marine Biological Association and of Mr Elmhirst, the director of the Millport Laboratory.

A considerable experience on the East coast of Scotland has shown that many species, particularly of Trematodes, common in certain regions along the western shores of Europe never occur in the fishes obtained from the Scottish part of the North Sea. For instance *Peracreadium genu* (Rud.), *Peracreadium commune* (Olss.), *Lebouria alacris* (Lss.), *Helicometra pulchella* (Rud.), *Prosorhynchus crucibulum*

(Rud.), and *Prosorhynchus aculeatus* (Odhn.) have never been met with. Five of these species are plentiful on the Norwegian coast and at least two have been obtained from the coast of Belgium. The reason for their absence from the Scottish coast is to be found in the fact that their hosts are absent, in some cases the final host, in others the intermediate. *Labrus berggylta*, the chief and almost the only final host of the *Perucreadium* species, does not occur on the East coast of Scotland and probably in this case the absence of the parasite is correlated with the absence of its definite host. The same applies to *Lebouria alacris* (Lss. nec. Nicoll 1909), which occurs only in the *Labridae*. This can hardly be the case with *Helicometra pulchella*, however, for it parasitizes many hosts, some of which are not uncommon in the North Sea. Here we probably have to reckon with the absence of the intermediate host. In the case of the *Prosorhynchus* species, I have not had an opportunity of examining a sufficient number of congers, the specific host of the parasite, to express any opinion.

It was partly with the view of gaining some information on this matter that the work at Millport was undertaken. The six species above mentioned were obtained there, mostly in great abundance, and are now recorded for the first time from British fishes. An attempt (Nicoll and Small, 1909) was made to discover the larval forms, in the course of which several of the common invertebrates were examined, but without success.

My main object was to examine several species of fish which could not be readily obtained from St Andrews Bay and to institute if possible a comparison between the parasitic fauna of the East and West coasts. As to the latter, the insufficiency of the material hardly admits of a just comparison, although in a certain proportion of cases such a comparison is not altogether impossible. As at St Andrews most of the widely distributed European species were met with; in many cases they are recorded here from new hosts. Most of these forms, *e.g. Hemiurus communis* (Odhn.), *Hemiurus lühei* (Odhn.), *Derogenes varicus* (Müller), *Zoogonoides viviparus* (Olsson), *Lecithaster gibbosus* (Rud.) and *Leioderma furcigerum*[1] (Olss.) occur in fishes both from deep and inshore water.

The equally widely-distributed *Podocotyle atomon* (Rud.) occurs only in littoral fishes. On the East coast several hundred deep-water

[1] The distribution of this species, however, is possibly not so widespread as might have been expected, for I have failed to obtain it on the South coast of England (Aug.—Sept. 1909).

fishes have been examined without a single specimen of that parasite being obtained, whereas almost all the littoral fishes are infected with it. In the enclosed waters of the Firth of Clyde, however, additional hosts in the shape of *Gadus virens*, *Gadus pollachius* and *Pleuronectes limanda* presented themselves. These were all young fish and were captured close inshore. Whether or not they rid themselves of the parasite when they leave the inshore waters and grow to maturity I have not been able to ascertain, but it certainly has not been found in any of the few adult specimens of saithe or pollack which I have had an opportunity of examining. From this the conclusion which may with justice be drawn, is that *Podocotyle atomon* is essentially an inshore form and that its cercaria encysts in some littoral invertebrate. In connexion with this species another point which I was enabled to confirm, is the fact that although plentiful in the salt-water stickleback (*Gasterosteus aculeatus* var. *trachurus*) it does not occur in the fresh-water variety (var. *gymnurus*).

Of the 29 species of fishes obtained, 17 had already been examined on the East coast, seven have since been examined, while the remainder were only met with on the West. The latter however were mostly isolated individuals and the only typically western fish which was examined in any number was *Labrus berggylta*. Elasmobranchs were not dealt with. Amongst the Teleosteans only two Lophobranchs (*Syngnathus acus* and *Nerophis lumbriciformis*) were examined and they yielded no parasites.

Infection on the whole reached almost the same degree as on the East coast. 80 % of the total number of fish examined were infected with helminths of one or more kinds. Most numerous amongst these were Nematodes which were present in 76 %, Trematodes were found in 70 %, Cestodes in 46 % and Echinorhynchi in 13 %. In the case of Nematodes however, in only a little over 4 % were they found in the alimentary canal. Almost a third of the fishes harboured immature or larval Nematodes in their body cavity or encysted in the peritoneum. The great preponderance of Cestode infection was in the form of scolices, chiefly *Scolex polymorphus*, which occurred in 17 %. Only about 6 % were infected with adult tapeworms. This agrees very closely with the figures found at St Andrews and we may therefore take it that tapeworms are the most uncommon form of parasite in marine Teleostean fishes. This has also been noted by Linton (1910) in the case of American fishes.

By far the commonest parasite was undoubtedly *Podocotyle atomon*

which occurred in nearly a quarter of all the fishes. It was especially frequent in the gadoid fishes, to the extent of nearly 40 %. Next to *Podocotyle atomon, Zoogonoides viviparus* and *Ascaris clavata* were the commonest parasites, each occurring in over 16 %, the former especially in the PLEURONECTIDAE (61 %) the latter in the GADIDAE (65 %).

Altogether 22 species of Trematodes were obtained. This compares favourably with St Andrews where over a much longer period only 25 species were met with; and with the Northumbrian coast where Miss Lebour (1908) has determined the presence of 22 species of digenetic Trematodes. From these three localities a total of 40 species has now been recorded.

SYSTEMATIC SECTION.

TREMATODES.

Sub-family I. ALLOCREADIINAE Looss 1899.

Next to *Allocreadium* Looss *sens. str.*, *Peracreadium* Nicoll 1909 appears to be the most typical genus of this sub-family. It diverges from the fundamental type in the enormous development of the cirrus-pouch and in the extensive distribution of the yolk-glands. It resembles *Allocreadium* in almost every other particular. The main lines along which, in the sub-family, variations from the type proceed, are in the direction of (1) the displacement of the genital aperture; (2) the lengthening or shortening of the cirrus-pouch; (3) the lobing of the ovary; (4) the more extensive distribution of the yolk-glands and (5) the growth of filaments on the ova.

In the first direction *Podocotyle* (Duj.) Odhn. 1904 represents the extreme and it also presents a transition stage in respect of the ovary, cirrus-pouch and occasionally of the yolk-glands. The position of the genital aperture distinguishes the genus from all the other genera but in *Lebouria* Nicoll 1909 we have a well-defined intermediate stage in which the displacement has proceeded only a very short distance. There is not wanting evidence, moreover, in several as yet imperfectly known species to indicate that more intermediate forms exist. With regard to the cirrus-pouch *Allocreadium, Helicometra* Odhn. 1902 and *Lebouria* represent the typical short, plump form, although in the last named there has entered a modification of the pars prostatica which is met with again in *Podocotyle*. This genus and *Cainocreadium* Nicoll 1909 are the intermediate stages towards the extreme development in *Peracreadium*. The typical form of the ovary must be regarded as

round, with entire margin. The first modification of this is apparent in *Podocotyle* with its characteristic trilobate ovary. In *Cainocreadium* the lobes, still three in number, are more pronounced, while finally in *Helicometra* the number may be increased to five. The increasing distribution of the yolk-glands can be traced very distinctly from *Allocreadium* through *Podocotyle* and *Lebouria* to *Peracreadium* in which it attains its maximum. Under normal circumstances *Podocotyle* agrees with *Allocreadium* in having the yolk-glands limited by the level of the ventral sucker, but as I have shown (1909 *a*, p. 452), in certain specimens of *Podocotyle atomon* (Rud.) an asymmetrical group of follicles makes its appearance in front of the ventral sucker on the right side. Exactly the reverse condition will be shown later to occur in a species of *Lebouria* where the yolk-glands normally extend in front of the ventral sucker, but in some cases are entirely absent on one side in front of the sucker. The eggs in most *Allocreadiinae* show a distinct thickening of the shell at the anopercular end. Occasionally this may be so marked as to form a slight knob, but it is only in *Lebouria* that this assumes any size and becomes at all frequent. In many cases an unmistakable spine is formed, but this is by no means constant, for a large number of the ova have no spines and even hardly a trace of a knob. That the condition in *Lebouria*, however, is the forerunner of the filaments in *Helicometra* there can hardly be any doubt, unless, perchance, evolution has proceeded in the reverse direction.

There is thus no direct line of development through these six genera and the facts seem to point to their origin from a hypothetical common type, closely resembling *Allocreadium*. This supposition is strengthened by the probability that it was from some such form that the nearly-related sub-families *Lepocreadiinae*, *Stephanochasminae*, and possibly *Echinostominae*, also arose. The position of the testes and ovary and the uniform restriction of the yolk-glands behind the ventral sucker (except in a few genera of *Echinostominae*) are remarkably constant throughout these sub-families.

Genus i. *Peracreadium* Nicoll 1909.

Species 1. *Peracreadium genu* (Rud. 1819).

This species was found frequently in considerable numbers in the rectum of *Labrus berggylta* and usually in association with *Helicometra*

pulchella (Rud.). It is a species which appears to be almost exclusively confined to this particular host; it certainly did not occur in any other fish at Millport. The best existing description of the form is that of Odhner (1902, pp. 497—9, Pl. 33, fig. 3). It has not hitherto been met with in British waters and appears to be entirely absent on the East coast.

The body has an elongated shape, somewhat flattened, but showing considerable thickness in the region of the ventral sucker. The colour is neutral grey, forming a striking contrast to the rich brown of *Helicometra pulchella*, a difference which renders easy the separation of the two species when occurring together in the same host.

All my specimens are adult and measure 1·5—2·4 mm. The average length is 2 mm. and all the following measurements will refer to a specimen of that length. The greatest breadth, at the level of the ventral sucker, is 0·6 mm. The breadth diminishes gradually towards each end.

The oral sucker is globular and has a diameter of 0·2 mm., *i.e.* $\frac{1}{10}$th of the body length. The ventral sucker is situated at a distance of 0·8 mm. ($\frac{2}{5}$ths of the body length) from the anterior end. It is transversely oval and measures $0{\cdot}32 \times 0{\cdot}355$ mm. It is therefore less than twice as large as the oral sucker and about $\frac{1}{6}$th of the body length.

The prepharynx is very short. The pharynx is large and round, measuring 0·13 mm. both in length and breadth. The somewhat rectangular shape noted by Odhner was not observed in my specimens, except occasionally in the living state. The oesophagus is a trifle shorter than the pharynx, having a length of 0·11 mm. The diverticula extend quite to the posterior end of the body.

The excretory vesicle is a simple one, reaching forward to the level of the anterior border of the anterior testis.

The genital aperture is median and is situated exactly at the level of the intestinal bifurcation. The cirrus-pouch is of great length and extends back to the level of the ovary. In young specimens it is usually not quite as long. Owing to its length, the characteristic club shape is not so well marked as in other members of the *Allocreadiinae*, the thickness being almost uniform. It contains a highly convoluted vesicula seminalis, a distinct though small pars prostatica, and a long slightly convoluted ductus ejaculatorius. In many specimens the cirrus was exserted and of great length. In not a few it was found inserted into the vagina of the same individual. This appears to be of rather frequent occurrence in this genus, for Olsson (1868) figures a specimen

of *P. commune* showing the same condition. It is suggestive of self-fertilisation.

The testes are situated in the posterior third of the body, the post-testicular space being $\frac{2}{9}$ths of the body length. They are always directly tandem and contiguous. The shape is somewhat irregular but approaches a transverse oval, the breadth being always greater than the length. In size they vary considerably but the longer diameter is usually about $\frac{1}{8}$th of the body length.

The ovary is situated immediately in front of the anterior testis, generally separated from it by the yolk-reservoir. It is on the right side of the middle line, of globular shape and somewhat smaller than the testes. The receptaculum seminis is of large size and lies slightly behind and dorsal to the ovary. It is in intimate connexion with the oviduct, there being no intervening receptacular duct. Laurer's canal is given off directly from the receptaculum.

The yolk-glands are unusually voluminous and of rather characteristic distribution. They fill up the post-testicular space and the lateral margins of the body as far forward as the posterior border of the ventral sucker, overlapping the testes and ovary to a slight extent. At the level of the ventral sucker they are entirely absent on each side but they again become voluminous in the neck, forming a continuous wedge-shaped mass in front of the ventral sucker, and extending forward to the level of the posterior border of the pharynx. The follicles are of moderate size, about 0·04 mm. in diameter.

The uterus is short and never contains more than 30 ova. The vagina is well marked and begins about the posterior border of the ventral sucker. The ova are rather large and broad, light yellow in colour and the shell is slightly thickened at the anopercular pole. The size varies considerably, *i.e.* 0·080—0·088 mm. in length and 0·044—0·056 mm. in breadth. The average size is 0·0845 × 0·051 mm. The rather great amount of variation in the breadth may possibly be due to the fact that the eggs are not completely circular in section but are flattened slightly from side to side.

Species 2. *Peracreadium commune* (Olsson 1868).

This species was also confined to *Labrus berggylta*, occurring along with, but much less frequently than *Peracreadium genu*. Only two specimens were obtained altogether. It bears a very close resemblance

to *P. genu*, but is not unreadily distinguishable from it by the difference in the distribution of the yolk-glands.

The two species are about the same size but *P. commune* is broader and flatter than *P. genu*. The colour has a tinge of brown in it. The suckers have much the same size and relative position but the ventral sucker is distinctly more oval than in *P. genu*. In a 2 mm. specimen it measures 0·22 × 0·31 mm. The pharynx presents one of the distinctive features of the species. It is almost fusiform in shape and much more elongated than in *P. genu*. Its size is 0·12 × 0·08 mm. The oesophagus is usually only about half the length of the pharynx. The diverticula extend to the posterior end.

In respect of the genitalia the two species are practically identical. The genital aperture has the same situation and the cirrus-pouch the same extent. The latter is possibly a little more dilated at its posterior end. The testes and ovary are decidedly smaller than in *P. genu* (about $\frac{2}{3}$rds the size). It is to the yolk-glands that we must look for the most distinctive feature of the species. Their extent and distribution are almost exactly the same as in *P. genu*, except that they are not interrupted at the ventral sucker. From the small amount of material at my disposal I am not able to confirm Odhner's observation that this is a constant feature of difference between the two species, and in view of the amount of variation in the yolk-glands which is possible in other *Allocreadiinae*, it is not to be denied that the two species might verge into each other. Taken, however, with the other less striking specific features, namely the elongated pharynx, the oval ventral sucker and the small ovary and testes, it forms a reliable enough distinguishing feature.

According to Odhner the ova appear to be much narrower than in *P. genu*, but that is not the case in my specimens.

Genus ii. *Lebouria* Nicoll 1909.

Species 1. *Lebouria varia* n. sp. = (*Lebouria*) *alacris* (Lss.) Nicoll 1909.

The doubt with which I regarded the identity of this form with *Distomum alacre* Lss. has now been resolved into a certainty that they are distinct. The differences are, however, not by any means striking owing to the rather considerable amount of variation which occurs.

The species is almost exclusively confined to *Callionymus lyra* in which it occurs fairly frequently, but rarely in number exceeding half a dozen. It appears to be distributed round the whole of the British

coast for I have obtained it at St Andrews and also at Plymouth. It has however not been recorded from the East coast of England. The only other host in which I have found the species is *Pleuronectes platessa*, in which immature specimens were obtained twice. Its occurrence in this host is rare and is possibly fortuitous. The two fishes have four other parasites in common, and thus it is not altogether unexpected to meet a frequent parasite of the one occasionally in the other.

The specimens obtained in the Clyde were all immature but I have since collected a number of mature individuals from the South coast. The following description will be made mainly from the latter.

The species has the typical Allocread shape, broadest at the ventral sucker, tapering gradually towards the posterior end, which is blunt, and more rapidly towards the anterior end. The colour is dull greyish yellow. The length of egg-bearing specimens varies from 1·25—1·75 mm. the average being 1·5 mm. The greatest breadth is about $\frac{1}{3}$rd of the length. The length 1·25 mm. seems to represent the maturity size, for quite a number of specimens were observed of sizes from 1·1—1·2 mm. in which egg production had not yet begun. The smallest specimen measured 0·32 mm. and the cereasia is evidently, therefore, of very small size.

The cuticle is unarmed and there are numerous subcuticular glands especially on the ventral surface anteriorly.

In an average specimen of 1·5 mm. length to which all the following measurements refer the oral sucker is subterminal and globular, with a diameter of 0·18 mm. The ventral sucker is transversely oval and may be raised on a pedicle in certain states of extension, as is the case to a greater or less extent with all the members of the sub-family. It is situated a little more than $\frac{1}{3}$rd of the body length from the anterior end (0·56 mm.). Its diameters are respectively 0·29 mm. and 0·35 mm. The diameter of the oral sucker is thus about $\frac{1}{8}$th of the body length and the diameters of the ventral sucker $\frac{1}{5}$th and $\frac{1}{4}$th respectively. The ratio of the transverse diameter of the suckers is very nearly 1 : 2.

There is a short prepharynx and a large pharynx, the diameter of which is more than half the diameter of the oral sucker. It is almost globular, the breadth being usually a little greater than the length (0·10 × 0·11 mm.). The oesophagus is short, being always shorter than the pharynx, about 0·06 mm. The intestinal diverticula diverge widely and terminate a little beyond the testes and thus at an appreciable distance from the posterior end of the body.

The excretory vesicle is a short simple sac, not extending further forward than the level of the anterior testis.

The genital aperture is situated a little in front of the intestinal bifurcation and is displaced somewhat to the left of the middle line. This would appear to be the characteristic position in the genus *Lebouria*. The cirrus-pouch is club-shaped, with a slight bend, and extends on to the dorsum of the ventral sucker but does not reach further back than the centre of the latter. It encloses a convoluted vesicula seminalis, much resembling that of *L. idonea*. The ductus ejaculatorius is short and almost straight, but occasionally it is somewhat convoluted. As in *L. idonea*, a pars prostatica is not distinctly differentiated, although prostatic cells are present. The testes are situated a little behind the middle of the body. They are placed invariably contiguous and obliquely, the anterior testis being always a little to the left. In only an occasional immature specimen were the testes directly tandem; in adults they were always oblique. No instance of amphitypy was observed. This oblique position of the testes would appear to be another characteristic of the genus for it occurs also in *L. alacris*, *L. obducta* and also, though not invariably, in *L. idonea*. They are of irregular ovoid shape, frequently approximating to a rounded triangular outline. The posterior testis is not uncommonly somewhat heart-shaped, with the apex directed backwards. They have never the transverse oval shape that commonly occurs in *L. idonea*. Their average diameter is about 0·2 mm. or a little less than $\frac{1}{7}$th of the body length. The post-testicular space (*i.e.* space between posterior testis and end of body) is 0·28 mm. in length, that is, nearly $\frac{1}{5}$th of the body length or somewhat less than one and a half times the diameter of the testis.

The ovary is situated midway between the anterior testis and the ventral sucker. The position, however, varies with the state of the animal, so that it may at one time be contiguous to the anterior testis, at another almost immediately behind the ventral sucker. Normally it is separated from the latter by a loop of the uterus and from the testis by the receptaculum seminis or occasionally the yolk-reservoir. It invariably lies to the right of the middle line and is a globular body with entire margins and a diameter of 0·105 mm.

The receptaculum seminis is pear-shaped and is situated on the same level as the anterior testis and contiguous with it as well as with the posterior testis and the ovary. Occasionally it lies almost dorsally to the ovary, as occurs in *L. idonea*. It is usually of considerable size.

Laurer's canal is given off directly from the receptaculum seminis and is of no great length. The shell-gland lies a little to the left of the ovary, between it and the yolk-reservoir, which is situated almost in the middle of the body.

The yolk-glands are very voluminous especially in the posterior half of the body. Their disposition is mainly lateral but they extend well to the inner side of the intestinal diverticula. They completely fill the post-testicular space. Further forward they overlap the margins of the testes and ovary to a variable extent. At the level of the ventral sucker they are as a rule sparse and may even be absent. In front of the sucker they increase again but are much less voluminous than in the posterior region. The glands from the two sides rarely if ever cross the middle line to unite in front of the ventral sucker as they do in *L. alacris*. Occasionally however a few stray follicles may be observed dorsal to the ventral sucker or cirrus-pouch. The anterior limit of the glands is subject to some slight variation. In most cases they stop a little behind the posterior border of the pharynx, but in a few specimens they reach the pharynx, in others they terminate at the level of the intestinal bifurcation. This, however, does not represent the full extent to which variation may proceed, for in at least one case the glands were entirely absent on the left side in front of the ventral sucker, although they were present to the normal extent on the right. The follicles are usually of large size with a diameter of 0·055 mm.

The uterus is very restricted in extent, being closely packed between the ventral sucker, the ovary and the anterior testis and containing only a small number of ova, never exceeding 20 in my specimens. The ova are almost exactly elliptical except that the opercular pole is blunted, almost flattened in fact. At the anopercular pole the shell is thickened, forming a slight knob but not nearly so pronounced as in *L. idonea*. They are of considerably larger size than in the latter species being 0·085—0·092 mm. in length and 0·038—0·051 mm. in breadth. The average size is about 0·088 × 0·045 mm.

This species must for the present be regarded as peculiar to *Callionymus lyra*. Its occurrence in *Pleuronectes platessa* can only be considered as accidental or at least very rare.

Species 2. *Lebouria alacris* (Looss 1901).

What I must now regard as the species actually described by Looss, was met with only once at Millport in *Labrus berggylta*. On the

South coast, however, I have obtained the species in considerable numbers from various LABRIDAE. It is not difficult to distinguish from *Lebouria varia* in the living state, but when preserved the differences are less marked and can be made out only by careful examination. At first sight the obvious difference in the disposition of the yolk-glands is striking, but beyond that there is little to go upon. On close inspection, however, one or two other differences will appear.

This species has much the same shape and appearance as *L. varia* but is distinctly lighter in colour, a fact which may be attributed to the less density of the yolk-glands and to the more delicate texture of the body in general. It is also much smaller than that species, being as a rule only about $\frac{2}{3}$rds its size. The specimens from the South coast were all mature and measured 0·8—1·45 mm. in length. Looss's specimens therefore were of maximum size (1·5 mm.). The average of my specimens is almost exactly 1 mm. The greatest breadth at the level of the ventral sucker is 0·46 mm. or rather less than half the length. The oral sucker has a diameter of 0·155 mm. and is thus between $\frac{1}{7}$th and $\frac{1}{6}$th of the body length. The ventral sucker is transversely oval and measures 0·22 × 0·26 mm. Its greater diameter is therefore a little more than $\frac{1}{4}$th of the body length. The ratio of the transverse diameter of the suckers is approximately 3 : 5. The ventral sucker is situated at a distance of 0·46 mm. from the anterior end and it is thus considerably nearer the centre of the body than is the case in *L. varia.*

There is a short prepharynx followed by a large pharynx measuring 0·077 × 0·066 mm. It is therefore absolutely smaller than that of *L. varia,* but proportionally it is actually of much the same size. The oesophagus as a rule is slightly longer than the pharynx, 0·09 mm. in length. The excretory vesicle has the same extent as in *L. varia.*

The genital aperture has also the same situation although it is perhaps a trifle further forward. The cirrus-pouch appears shorter owing to the backward displacement of the ventral sucker. Its posterior end only extends a short distance beyond the anterior border of the sucker. The disposition of the genital glands is almost precisely the same as in *L. varia.* The testes however are a little further back so that the post-testicular space comprises only $\frac{1}{6}$th of the body length. The testes in addition are relatively smaller being only 0·12 mm. in diameter so that again the post-testicular space is equal to about one and a half times the diameter of a testis. The diameter of the ovary is about 0·085 mm.

In the yolk-glands we find the chief distinguishing character of the species. In the posterior part of the body they are exclusively marginal and rarely extend to the inner side of the intestinal diverticula. In the post-testicular space they do not unite and thus leave a vacant space behind the posterior testis. There is however occasionally a certain amount of proliferation. In no case do they overlap the testes or ovary. They are always continuous at the level of the ventral sucker and immediately in front begin to proliferate to such an extent that they extend right across the body. This extension however is entirely under the dorsal surface. They thus form a dorsal layer which extends from over the ventral sucker to well in front of the intestinal bifurcation. The anterior limit of the marginal follicles is about the level of the middle of the pharynx. Sometimes they barely reach the pharynx.

The uterus is confined in the same way as in *L. varia* and never contains more than 20 ova. The latter are a trifle shorter and broader but have otherwise the same shape. They measure 0·081—0·088 mm. in length by 0·041—0·054 mm. in breadth: average 0·083 × 0·049 mm.

To sum up the differences between the two foregoing species we find that the yolk-glands are differently disposed, that the ventral sucker is nearer the middle of the body in *L. alacris* and that the suckers are proportionally somewhat larger. The testes and post-testicular space are somewhat smaller and the oesophagus is longer. In addition *L. alacris* is a much smaller species than *L. varia* and begins to produce ova at a considerably smaller size (0·8 mm. as contrasted with 1·25 mm. in the case of *L. varia*).

With the addition of these two species the original definition (1909 *a*, p. 450) of the genus *Lebouria* requires slight modification as follows: Genital aperture slightly displaced from the middle line towards the left. Testes usually oblique, the anterior one being to the left. Ova measuring 0·065—0·09 × 0·035—0·05 mm.

Genus iii. *Podocotyle* (Duj.) Odhner 1904.

Species 1. *Podocotyle atomon* (Rud.) 1802.

This species has already been exhaustively described by Odhner (1904, p. 320), Lebour (1907, p. 36) and myself (1907, p. 73, 1909 *a*, p. 451, 1909 *b*, p. 6). It is the commonest parasite of inshore fishes. At Millport it was found in *Cottus scorpius, Cottus bubalis, Pholis gunnellus, Gadus pollachius, Gadus virens, Pleuronectes limanda* and *Pleuronectes platessa.*

Genus iv. *Helicometra* Odhner 1902.

To this genus five species have now been referred, viz. *H. pulchella* (Rud.), *H. fasciata* (Rud.), *H. sinuata* (Rud.) *H. mutabilis* (Stossich 1902) and *H. flava* (Stossich 1903). Two other species described by Stossich, namely *Distomum gobii* and *D. labri*, also belong to this genus, but the latter was found by Odhner (1902) to be identical with *H. pulchella*. The identity of all the species, however, can hardly be regarded as beyond question. The genus *Helicometra* as defined by Odhner (1902, p. 161) forms a distinct systematic unit, the outstanding feature of which, separating it from all other *Allocreadiinae*, is the filamented condition of the ova. At the same time it is unfortunate that the genus should have been founded on a type-species which is, strictly speaking, still a *species inquirenda*, for Odhner failed to elucidate the anatomy of *Distomum pulchellum* Rud. on a point which may prove to be of the greatest importance, namely the condition of the testes. Odhner's identification (1902, p. 160, note 2) as the type specimens of *Distomum pulchellum* Rud. certain specimens in the Berlin collection labelled "*Distomum Sp. Labrus cynaedus* Neapel. Coll. Rudolphi" is itself open to dispute, although the probabilities are very much in its favour. Still more questionable is his conclusion that *Distomum labri* Stossich (1887) is identical with the form which he describes as *H. pulchella* (Rud.). It is almost inconceivable that Stossich could have described the testes as multilobate, with five to six lobes, had such not been the case. Yet Odhner examined one or more of Stossich's specimens and found them to agree with his own, in which the testes are entire (*ganzrandig*), the statement of which fact being underlined. It is evident that some discrepancy has entered here, the significance of which Odhner either ignored or failed to grasp. A possible explanation will be offered in the following notes.

By the kindness of Professor Monticelli of Naples, specimens of *Distomum gobii* (from *Gobius jozo*) and *Helicometra mutabilis* (from *Anguilla vulgaris*) from Stossich's collection were placed at my disposal. Specimens of *Distomum labri* and *Helicometra flava* were not available. From an examination of this material I have not the slightest doubt that the first two species are identical with each other and with the form which I am describing here as *Helicometra pulchella*. *Distomum labri* is also certainly identical with these and there is more than a

suspicion that the same might be said about *Helicometra flava*. Three at least, therefore, of Stossich's species are synonymous with each other and probably also with *Helicometra pulchella* (Rud.). I have as yet, unfortunately, not been able to examine specimens from Odhner's collection.

My specimens do not agree with Odhner's description, but correspond much more closely with Stossich's *Distomum labri* (1887). Considering that Rudolphi's and Stossich's specimens were collected in adjacent localities it is not improbable that they are identical, but that the form obtained by Odhner from the northern Labridae is the same species is somewhat doubtful.

Species 1. *Helicometra pulchella* (Rud.) 1819.

Synonyms. *Distomum gobii* Stossich 1883.
Distomum labri Stossich 1883.
Loborchis mutabilis Stossich 1902.
Helicometra mutabilis Stossich 1903.

This form occurred in the lower part of the intestine of *Labrus berggylta* and *Conger conger*, in about 60 % of the former and 33 % of the latter. It is usually met with in moderate numbers up to about twenty in a single host. It is readily distinguished in the intestinal contents by its rich brown colour. Young specimens, however, are comparatively colourless.

Its shape is like that of most ALLOCREADIINAE but the post-acetabular part is much flattened and leaf-like, with a fairly uniform breadth. The neck narrows gradually forwards from the ventral sucker, but is, as usual, capable of great extension. The length of adult specimens is 1·3—4·3 mm. the average being 2·5 mm. Those from the conger were particularly large. In the wrasse they never exceeded 3 mm. and in other fishes from which I have obtained the species elsewhere the average size was usually about 2 mm. It appears to attain maturity at a size of about 1·25 mm. but specimens containing a few malformed eggs have been found as small as 1·1 mm.

In an average specimen of 2·5 mm. length, the maximum breadth at the ventral sucker is 0·83 mm. Both suckers are globular. The ventral sucker retains this shape, no matter how the animal is killed or pressed. In this respect it differs from other ALLOCREADIINAE in which, during life, the ventral sucker may appear globular, but, on

death, it always assumes a transverse oval shape. It is, moreover, more sessile than usual. The oral sucker has a diameter of 0·23 mm. or rather less than $\frac{1}{10}$th of the body length. The diameter of the ventral sucker is 0·35 mm. so that the ratio is 2 : 3. The ventral sucker is situated a little more than $\frac{1}{3}$rd of the body length (0·9 mm.) from the anterior end. There is a very short prepharynx and the almost globular pharynx measures about 0·1 mm. in diameter. The oesophagus is barely half as long again as the pharynx and the bifurcation takes place about midway between the pharynx and the anterior border of the ventral sucker.

The excretory vesicle is simple and extends forward to the level of the ovary.

The testes are situated just behind the middle of the body, always directly tandem and contiguous. The post-testicular space comprises about $\frac{1}{7}$th of the body length, but it varies from $\frac{1}{6}$th to $\frac{1}{10}$th. The posterior testis is always the larger of the two. They are confined between the intestinal diverticula and are never overlapped by yolk-glands. The outline of each testis is irregularly lobed, the lobing being in the majority of cases well-marked and unmistakable. The number of lobes is not constant, but there are usually from five to seven. In addition each lobe is frilled or sub-divided into smaller lobules. The lobing is entirely lateral and posterior. The anterior surface from which the vas efferens issues is comparatively even. The anterior testis is less lobulated than the posterior. The condition is most pronounced in young specimens; in old specimens the lobes appear to get pressed out by distension so that on a cursory examination the outline looks merely uneven. On careful inspection, however, the traces of the lobes can always be made out. Even in the largest specimens the testes are frequently as much cut up as in the younger ones. In specimens which have been incompletely fixed the outline of the testes is not uncommonly indefinite and under such circumstances it might be regarded as unlobed. The breadth of each testis is about 0·37 mm. The length of the anterior one is slightly less; that of the posterior rather more.

The genital aperture is situated in the middle line, in front of the intestinal bifurcation and near the middle of the oesophagus. The cirrus-pouch is short and narrow. It is straight or very slightly curved and extends back to the anterior border of the ventral sucker, or a little beyond it. It contains a convoluted vesicula seminalis and a moderately long ductus ejaculatorius which is also slightly convoluted.

A distinct pars prostatica appears to be absent as in the case of *Podocotyle* and *Lebouria*, but a number of gland-cells are present within the cirrus-pouch, which discharge irregularly into the ductus.

The ovary is situated immediately in front of and contiguous to the anterior testis and is much smaller than it. It may be either in the middle line or a little to the right or left. It is irregularly lobed, there being usually three or four main lobes each with an irregular outline. The anterior part from which the oviduct takes origin may also be considered an additional lobe. Most commonly its breadth greatly exceeds its length, but occasionally it is more compact. The receptaculum seminis is pear-shaped and of large size. It lies usually on the right side of the ovary, but not infrequently it is found on the left. From it a long Laurer's canal is given off. From the receptaculum the oviduct proceeds inwards to the ootype, receiving on its course the yolk-duct. The yolk-reservoir is small and lies in front of the ovary. The yolk-glands are extensive and have a definite and fairly constant configuration. Their greatest variation occurs in the neck. Here they usually stop short of the posterior end of the pharynx, but they may extend a little in front of this or terminate at the level of the middle of the pharynx. They are mainly lateral in position, following the course of the intestinal diverticula. They thus proceed inwards towards the intestinal bifurcation and in some cases the follicles unite in the middle line dorsally. In a few specimens there was a certain amount of proliferation in this region but in the great majority no union takes place. Behind the ventral sucker the follicles are entirely to the outer side of the intestinal diverticula, except at the level of the uterus where they overlap to a certain extent. At the level of the testes the yolk-glands never completely overlap the diverticula. In the post-testicular space their distribution is characteristic. They follow the outer border of the diverticula to their termination, turn there and follow the inner border of the diverticula to the posterior end of the second testis. No fusion of the follicles takes place in this space, the excretory vesicle always serving as a dividing mark between those on either side.

The uterus is confined to the space between the ovary and the ventral sucker. The condition of the ova gives it a configuration which is peculiar to the genus and unique amongst the ALLOCREADIINAE. In mature specimens it is arranged in the form of a spiral of three to five loops, superimposed on each other, and each a little in advance of the one below. Each ovum at its anopercular end is provided with a long filament, which is six to eight times as long as the ovum. These

are directed backwards and become intertwined into a spiral. Together they form a continuous thread on which the ova have the appearance of being stuck. The ova are all situated on the inner side of the spiral and at equal distances apart. In the vagina they tend to become separated from the spiral and are probably deposited singly although I have observed a complete spiral extruded. The filaments are not motile.

The ova number about fifty. They are dark brown in colour, with a moderately thick shell and have a characteristic shape. From the lateral aspect, which is the view usually obtained, they appear comma-shaped with the broad end pointing forward and the narrow end tapering off into the filament. One surface is therefore convex, the other concave, and the former is that which lies along the spiral. When turned over on to one or other of these surfaces the outline appears ovoid, the broad end again being anterior. In size they vary considerably. The length is 0·063—0·084 mm.; the transverse breadth 0·032—0·037 mm. and the breadth from convex to concave surfaces 0·027—0·33 mm. at its maximum. The average of these, to which the majority approximated, is $0{\cdot}073 \times 0{\cdot}033 \times 0{\cdot}030$ mm. No segmentation takes place before the deposition of the eggs.

It is obvious that the foregoing description does not agree with that of Odhner (1901 and 1902) but that it agrees very closely with Stossich's description of *Distomum labri* (1887) and *Distomum gobii* (1883). The chief differences contained in Odhner's description are (1) the testes are entire and oblique; (2) the yolk-glands overlap the intestinal diverticula posteriorly, and form a continuous narrow band across the post-testicular space; (3) the genital aperture is just over the intestinal bifurcation and the cirrus-pouch extends to the middle of the ventral sucker; (4) the shape of the ovary is somewhat different and it is not directly in front of the testes. These differences, if constant, would be sufficient to constitute a difference in species. The probability, however, that they are not without the limits of variation induced me to examine carefully every individual specimen, amounting to nearly 200, in my collection. These were mostly obtained on the South coast of England and from such varied hosts as *Trigla pini*, *Gobius paganellus*, *Blennius pholis* and *gattorugine*, *Lepidogaster gouanii*, *Labrus mixtus*, *Ctenolabrus rupestris*, *Zeugopterus punctatus* and *Anguilla vulgaris* but a remarkable degree of uniformity was observed, and that they all belonged to one species there can be no question. In not one single case were the testes otherwise than strictly tandem; in almost every instance they

were distinctly lobed and in the exceptions, where distension had occurred, the lobing could be made out without much difficulty. The disposition of the yolk-glands in the post-testicular space was also always as I have described it. Upon these three features mainly the identity of the species depends. As already remarked, it is highly probable that the British form is identical with the Mediterranean form and therefore, with Rudolphi's original. The form described by Odhner, if errors of observation be excluded, may be regarded as a northern variety or species. The extreme degree of obliquity of the testes noted by Stossich in *Loborchis mutabilis* suggests that obliquity may be a possible variation in the species even although it has not been observed in my specimens.

Sub-family II. Stephanochasminae Looss.

Genus i. *Stephanochasmus* Looss 1899.

Species 1. *Stephanochasmus baccatus* Nicoll 1907.

About half a dozen immature specimens were found in the intestine of a single *Cottus scorpius*. Being immature they can hardly be identified with certainty, but the number of cephalic spines, namely 28 in each row, points almost unmistakably to *S. baccatus*. In two of the specimens there were only 27 in each row, but variation in the number to this extent appears to be found in all the species of *Stephanochasmus*. The length of the spines was 0·024—0·032 mm.

The specimens measure 1·25—2·05 mm. in length. The maturity size must be about 2·2 mm. In the largest specimen the oral sucker measures 0·17 mm. and the ventral sucker 0·22 mm. The neck being well extended the prepharynx is two and a half times as long as the pharynx, which measures $0{\cdot}13 \times 0{\cdot}10$ mm. The cirrus-pouch extends only a short distance behind the ventral sucker.

The encysted larva of this species was found under the skin in *Pleuronectes limanda* (Nicoll and Small 1909) and has later been found by Elmhirst at Millport in *Drepanopsetta platessoides*.

Species 2. *Stephanochasmus pristis* (Deslongch).

A few immature specimens of this species were taken from the pyloric coeca of a cod (*Gadus callarias*). Miss Lebour (1908) was the first to record the occurrence of this species in the cod, but it is not at all common. She met with it in only 2 %.

The length of my specimens is about 1 mm., the breadth 0·15 mm. The suckers are approximately equal with a diameter of 0·12 mm. the oral being if anything larger than the ventral. The latter is situated 0·36 mm. from the anterior end. The cephalic spines are in two rows of 18 each and each row forms a complete uninterrupted circle. The spines are comparatively stout.

Sub-family III. Lepocreadiinae Odhner 1904.

Genus i. *Lepidapedon* Stafford 1904 = *Lepodora* Odhner 1904.

Species 1. *Lepidapedon rachiaeum* (Cobbold 1858).

This species was found in considerable numbers in the intestine, usually towards the anterior end, of *Gadus pollachius* (55 %) and *Gadus virens* (45 %). Odhner has recorded it from *Gymnocanthus ventralis*, but in British waters it appears to be confined to the *Gadidae* and in particular to the two above-mentioned species together with *Gadus aeglefinus*. In *Gadus callarias* its place is taken by *Lepidapedon elongatum* (Lebour) and in *Gadus merlangus* by *Pharyngora bacillaris* (Molin). It has been fully described by Odhner (1904, pp. 332—7), Lebour (1908, pp. 59—60) and myself (1907, pp. 77—80).

Genus ii. *Pharyngora* Lebour 1908.

Species 1. *Pharyngora bacillaris* (Molin 1859).

Synonyms. *Distomum increscens* Olsson 1868, pro parte.
Pharyngora retractilis Lebour 1908.

The species on which the definition of the genus *Pharyngora* was founded by Miss Lebour was regarded by her as distinct from *Distomum bacillare* Molin, but that the two are identical there can be little doubt. From an examination of some specimens from *Gadus merlangus* which Miss Lebour was good enough to send me I have been able to assure myself that they are the same as those which I have obtained in large numbers from *Scomber scombrus* at Millport and elsewhere. Molin's original description (1859, p. 818), of the species is very incomplete, but it is accurate so far as it goes. It was redescribed and figured by Stossich (1887, p. 92, Pl. X, fig. 38) but two of the most important characters were omitted, namely the external vesicula seminalis and the cuticular spines. This is not surprising for the species is of great delicacy

and rapidly begins to macerate after the death of its host. Unless the parasite be obtained alive it is extremely difficult to discern the outline of the vesicula seminalis externa, and the spines are so minute that they might readily escape detection. Otherwise the resemblance is so great that there is little doubt Stossich was dealing with the same species[1]. The species described by Olsson (1868, p. 36, Pl. 4, fig. 83) as *Distomum increscens* is also, as already noted by Odhner (1904, p. 332, note 4), in part identical with *Distomum bacillare*.

Pharyngora bacillaris (Molin) is the commonest parasite of the mackerel and was found at Millport in over 80 %. That figure is probably above the normal, but it certainly occurs in well over 50 % on other parts of the coast. It was not met with in any other fish in the Firth of Clyde, but elsewhere it is met with frequently in *Cyclopterus lumpus*, *Capros aper* and not uncommonly in *Gadus merlangus*. The infection is generally in considerable numbers and in *Cyclopterus* it may be enormous.

All the specimens from Millport were immature or just beginning to produce ova, so the following description will be based on specimens obtained elsewhere.

It is a delicate and slender form, generally colourless except for the uterus, which has the usual brownish yellow colour. Older specimens frequently have a rusty brown colour. The specific name is rather apt, for it is elongated and of almost uniform breadth. Dorso-ventrally it is considerably flattened. In life its movements are comparatively sluggish, resembling in this respect those of *Lepidapedon rachiaeum*, and they are mostly of a forward and backward character. It shows less tendency than other Distomes to curl from side to side. This may be correlated with the reduction in size of the ventral sucker, and may also account for the fact that specimens always die straightened out.

The length of mature individuals is 2—4·5 mm. The maturity size appears to lie almost exactly at 2 mm., but an occasional specimen without ova was met with over this length. No smaller specimen, however, was seen with eggs. The breadth is very uniform. It is usually greatest at the level of the testes but there is little difference up to the level of the ventral sucker. In front of that it gradually narrows to the square-cut anterior end. The posterior end is slightly

[1] From the examination of some of Stossich's specimens, kindly sent me by Professor Monticelli, I have been able to make certain that Stossich was dealing with the same species as I am here describing.

rounded but comes to a point at the extreme tip. In an average specimen of 2·75 mm. length the maximum breadth is 0·36 mm.

The cuticle is entirely covered with minute scale-like spines, most closely set in the neck and becoming sparse towards the posterior end. There is no special cephalic armature. A distinctive feature, which has escaped the notice of previous observers, is the presence of a pair of large pigment patches, one on each side of the pharynx. They consist of a number of points of deep brown colour, considerably scattered and lying towards the anterior end of the pharynx. They are present even in the oldest specimens.

The oral sucker is practically terminal, although its aperture has a ventral inclination. Its uncommon shape has suggested to Miss Lebour the name for the genus. It differs from the ordinary globular sucker in having its posterior end drawn out and it appears as if somewhat constricted about its middle part. Moreover, the posterior pole, where the prepharynx joins on, is usually slightly introverted, forming a sort of papilla in the bottom of the sucker. In specimens which have died before being collected, a variety of appearances may present themselves. In many the wide-mouthed funnel shape, figured by Stossich, is seen and this in conjunction with other circumstances suggests that Stossich's specimens had been dead some time before he obtained them. This would account for the absence of external seminal vesicle, spines and pigment patches in his figure. Frequently the sucker is found so greatly retracted that it is drawn quite within the body and communicates with the exterior only by a minute aperture. In several cases, again, the pharynx is found completely enclosed in the sucker and in one or two cases it had been actually ejected through the mouth. All these appearances point to a comparatively enormous muscular development of the anterior part of the body, and this again may be correlated with the relative weakness of the ventral sucker and the rest of the body. The sucker presents the further peculiarity of having the ventral border of its aperture notched in the middle line. This is a constant feature.

Although the oral sucker has an elongated appearance its length is really not greatly in excess of its maximum diameter and frequently indeed it is less. In a 2·75 mm. specimen the maximum diameter is 0·21 mm. The ventral sucker is situated at a distance of 0·95 mm. from the anterior end and the neck is thus a little more than a third of the body-length. The sucker is circular and flat but it is capable of being raised to a small extent above the surface of the body. Its diameter is 0·16 mm. and the ratio to the oral sucker is very nearly 3 : 4.

The alimentary system is of a unique type. There is a distinct though short prepharynx. Its length is usually about a third of the length of the pharynx. Frequently it is less and occasionally it is nearly as long as the pharynx. I have never observed a specimen in which it was three times as long as the pharynx, as noted by Miss Lebour. This could only occur when the animal was alive and the anterior end in a state of hyper-extension. In this respect Stossich's figure shows the normal state. The pharynx is rather small but very muscular. It measures 0·145 × 0·105 mm. and is usually thickest at its posterior end. The oesophagus, though apparently of great length, is in reality very short. What has hitherto been described as the oesophagus, is, as first noted by Odhner (1904, p. 338), not a true oesophagus, but is actually the initial parts of the intestinal diverticula fused together. It is lined with ciliated epithelium continuous with that of the gut. The intestinal bifurcation, therefore, takes place actually in the intestine and not at the junction of the oesophagus with the diverticula. The whole of the tract between the pharynx and the bifurcation, however, is not of the same nature for there is a short oesophagus with the usual structure and not lined with intestinal epithelium. This comprises about $\frac{1}{7}$th of the total length from the pharynx to the bifurcation, which is 0·39 mm. The oesophagus therefore measures on an average 0·055 mm. and is thus about the same length as the prepharynx. The pseudo-oesophagus, as it has been termed by Odhner, is capable of considerable dilatation. The diverticula are of normal structure and extend quite to the posterior end of the body.

A converse type has already been described (Nicoll, 1909 *a*, p. 407) in the case of *Stephanophiala laureata* (Zed.) and some allied species, in which the intestinal bifurcation takes place actually in the oesophagus, so that the initial parts of the diverticula are morphologically portions of the oesophagus.

The excretory vesicle, described by Miss Lebour as extremely small, is on the contrary of great size. It is a long narrow sac stretching from the posterior end to well in front of the intestinal bifurcation. As far as can be made out, however, it is not invariably as large as this and it appears to be capable of a certain amount of contraction. An even larger excretory vesicle is met with in the allied genus *Lepocreadium*. In living specimens the main excretory tubules are very conspicuous especially in the neck. They are highly convoluted.

The testes are situated in the posterior third of the body, always directly behind each other. They are frequently contiguous, but never

pressed against each other. They may be separated by a small space occupied to some extent by yolk-glands. They are elongated oval in shape and of moderate size, the dimensions being 0·20 × 0·18 mm. In living specimens the outline is usually slightly crenated but this disappears on preservation. The post-testicular space is about $\frac{1}{7}$th of the body-length or equal to twice the length of a testis.

The terminal part of the male reproductive organs consists of a muscular cirrus-pouch, containing a simple vesicula seminalis, pars prostatica and ductus ejaculatorius. In addition there is a vesicula seminalis externa, lying free in the parenchyma outside the cirrus-pouch and connected with the internal seminal vesicle by a short duct. It is sac-like, elongated and a little larger than the internal vesicle. It usually lies immediately behind the cirrus-pouch. The internal vesicula seminalis is small and almost globular. It opens directly into the pars prostatica, which is also somewhat globular. The ductus ejaculatorius traverses the remaining part of the cirrus-pouch. It is usually slightly convoluted and of nearly uniform calibre but sometimes it appears almost straight. No spines are present in it and I have never seen it exserted. The cirrus-pouch extends behind the ventral sucker for a distance rather greater than the diameter of the sucker.

The ductus opens into a comparatively large genital sinus, which is constantly oval in shape and lies on the left anterior border of the ventral sucker. Its representation by Miss Lebour on the right side is apparently an oversight.

The ovary is situated immediately in front of the anterior testis and may be contiguous with it or separated by a short distance. It is displaced from the middle line slightly to the right side and is considerably smaller than either testis. It is almost heart-shaped in outline, the apex being directed towards the middle line and from it the oviduct issues. The shell-gland complex is of normal type, the oviduct, yolk-duct and receptaculum seminis all opening into the proximal end of the ootype, and from the junction Laurer's canal is given off. The ootype is short and the shell-gland is diffuse. The receptaculum seminis lies to the left of and somewhat dorsal to and behind the ovary. Usually it is small and pear-shaped but occasionally it is found greatly distended and almost globular.

The yolk-reservoir lies between the ovary and the receptaculum seminis. The yolk-glands are dense but somewhat circumscribed. They fill the whole of the post-testicular space, uniting there across the middle line, and extend forward along the sides of the body to the

level of the posterior end of the cirrus-pouch. They cover the intestinal diverticula and overlap the testes to a slight extent. The variation in the anterior limit of the yolk-glands is very small and they never reach the ventral sucker as is shown in Miss Lebour's figure of *Pharyngora retractilis*.

The uterus occupies the space between the ovary and the ventral sucker but it is poorly developed. It consists of only a few small convolutions and the ova lie freely in them. The ova rarely exceed 50 in number. They are of regular shape, with uniform shell slightly thickened at the anopercular pole. They vary greatly in size, the limits in length being 0·072—0·091 mm. and in breadth 0·042—0·053 mm. The average dimensions are about 0·081 × 0·047 mm.

The larva of this species was obtained from some material brought in by a coarse-meshed tow-net at Plymouth in August. It was quite free when found and there was no evidence to show that it had escaped from any of the numerous Copepods and larval crustacea included in the haul. A number of them were examined but no cysts could be found. The specimen measured about 0·4 mm. and there could be no mistake as to its identity. The peculiarly shaped oral sucker, the conspicuous pigment patches, the long oesophageal tube and the large excretory vesicle, extending well in front of the ventral sucker, all combine to render its identity practically certain.

From the foregoing it is evident that Miss Lebour's original definition of the genus *Pharyngora* requires modification in certain important points. The changes and additions which require to be made are as follows:

Lepocreadiinae of delicate structure and elongated body; pigment patches alongside the pharynx, persisting in the adult; prepharynx comparatively short, pharynx small, oesophagus short but continued by a long pseudo-oesophagus lined with intestinal epithelium; excretory vesicle greatly elongated; genital aperture on the left anterior border of the ventral sucker; genital sinus capacious, ovoid in shape; vesicula seminalis externa lying free in the parenchyma and not surrounded by any specialized membrane or glands; cirrus-pouch elongated, extending behind the ventral sucker; vesicula seminalis in terminal communication with the pars prostatica.

Type and only species *Pharyngora bacillaris* (Molin 1859). Habitat, intestine of marine fishes.

It is also apparent that, as already indicated by Odhner, it falls naturally into the sub-family Lepocreadiinae, but its inclusion

neeessitates some slight modifieation of the definition of that sub-family. It is more elosely related to *Lepocreadium* than to *Lepidapedon*, and it is only separated from the former by the shape of the oral sueker, the presenee of pigment patches, the short prepharynx and the presenee of the pseudo-oesophagus. The ehanges whieh might be suggested in the definition of the sub-family are as follows:

Ineludes forms of 1—6 mm. in length; ventral sueker feebly developed and usually smaller than the oral sueker; prepharynx usually elongated but may be short; initial parts of intestinal divertieula may be fused to form a pseudo-oesophagus; Laurer's eanal not arising direetly from the reeeptaeulum seminis.

The last-mentioned feature is a further point of distinetion between this sub-family and the Allocreadiinae, in which Laurer's eanal arises direetly from the distal end of the reeeptaeulum seminis.

Sub-family V. Fellodistominae Nicoll 1909.

Genus i. *Leioderma* Stafford 1904.

Synonym. *Steringophorus* Odhner 1904.

I have hitherto used the name *Steringophorus* for this genus, but Stafford's name has priority, although at the same time his definition is inadequate.

Species 1. *Leioderma furcigerum* (Olsson 1868).

This speeies is evidently not nearly so common in the Firth of Clyde as on the East eoast. It was found only in *Pleuronectes limanda* in 33 per cent. Taking all the *Pleuronectidae* examined, its oeeurrenee was only to the extent of 12 per cent. In my experienee it is twiee as common on the East eoast and aeeording to Miss Lebour it is four times as eommon on the Northumberland eoast. Its plaee in the Firth of Clyde appears to be taken to a large extent by the allied speeies *Leioderma cluthense*. On the South eoast of England it seems to be entirely replaeed by that species.

A very full aeeount of the form is given by Odhner (1904, pp. 305—310) and Miss Lebour (1908, pp. 53—55).

Speeies 2. *Leioderma cluthense* Nicoll 1909.

This speeies was found in every speeimen of *Pleuronectes microcephalus* and in no other host. A fairly eomplete deseription has already been published (Nieoll, 1909 *a*, pp. 472—475).

Sub-family VI. Zoogoninae Odhner 1902.

Genus i. *Zoogonoides* Odhner 1902.

Species 1. *Zoogonoides viviparus* (Olsson 1868).

Next to *Podocotyle atomon* this was the commonest species found at Millport. It occurred in *Pleuronectes limanda* (67 %), *Pl. microcephalus* (100 %), *Pl. platessa* (67 %), and *Callionymus lyra* (80 %). It is thus extremely frequent in each of these hosts. In a previous paper (1909 *b*, p. 16) I remarked that the species occurred in *Pleuronectes flesus* at Millport, but that was in error for *Pl. limanda*. The former fish was not examined at Millport. The number of British hosts of the species is therefore only eight. Six of these are flat fish so that the species is a typical Pleuronectid parasite, notwithstanding the fact that *Callionymus lyra* is probably the most frequent individual host.

It almost invariably occurs in large numbers, 100 or more, even in young fish. As a rule it is most numerous in the rectum but frequently it extends throughout the intestine.

It is described in detail by Odhner (1902), Miss Lebour (1908) and myself (1907).

Sub-family VII. (Derogeninae).

Genus i. *Derogenes* Lühe 1900.

Species 1. *Derogenes varicus* (O. F. Müller).

This occurred in the oesophagus and stomach of seven hosts, namely, *Cottus scorpius* (60 %), *Gadus callarias* (17 %), *Gadus merlangus* (100 %), *Gadus minutus* (50 %), *Gadus pollachius* (71 %), *Gadus virens* (14 %) and *Pleuronectes platessa* (17 %). Of these the last four are new British hosts. The total number of the latter is now 19, but this does not by any means exhaust the list, as I shall have occasion to show later. The species is the most widely distributed of all marine Trematodes. The infection in each host, however, rarely exceeds half a dozen.

Numerous descriptions of the species exist. The most recent are those by Odhner (1902), Johnstone (1907) and Miss Lebour (1908).

Sub-family VIII. Lecithasterinae Odhner 1905.

Genus i. *Lecithaster* Lühe 1901.

Species 1. *Lecithaster gibbosus* (Rud.) 1802.

This species was comparatively frequent. It was met with in the intestine of *Trigla pini*, *Gobius ruthensparri*, *Labrus berggylta*, *Gadus merlangus* and *Drepanopsetta platessoides*. With the exception of *Gadus merlangus* all these species are new British hosts of the parasite. In its wide distribution and in the fact that few specimens occur in each host, it resembles *Derogenes varicus*, but the numbers which occur are even much less than in the case of the latter species. In most of its hosts only a stray specimen occurs, but in *Gadus merlangus* quite a large infection may be met with. In *Drepanopsetta platessoides* also I found nearly a dozen specimens but they were all immature.

For a description see Odhner (1902), Lebour (1908), and Nicoll (1909 *b*).

Sub-family IX. Sterrhurinae Looss 1907.

Genus i. *Lecithochirium* Lühe 1901.

Species 1. *Lecithochirium rufoviride* (Rud.) 1819.

This species is confined to the conger-eel, in practically every specimen of which it is to be found. It is a stomach parasite and occurs in enormous numbers. It has already been recorded from Millport by Elmhirst and Martin (1910).

It is a well-known species and has been frequently described, most recently by Johnstone (1907, pp. 177—180, as *Distomum ocreatum* Molin) and Looss (1908, pp. 144—147).

Sub-family X. (Dinurinae Looss 1907.)

Genus i. *Lecithocladium* Lühe 1901.

Species 1. *Lecithocladium excisum* (Rud.) 1819.

This species also is confined to one host, *Scomber scombrus*, in the stomach of which it is frequently found, although never in such large numbers as is the case with the preceding species. The two are rather like each other in size, shape and colour but their internal structure is greatly different.

The last description is that by Looss (1908, pp. 131—2).

Sub-family XI. Hemiurinae (Lühe 1901) Looss 1907.

Genus i. *Hemiurus* (Rud. 1819) Looss 1907.

Species 1. *Hemiurus communis* Odhner 1904.

This species was found in the stomach of six different fishes, namely, *Labrus berggylta, Gadus callarias, Gadus minutus, Gadus pollachius, Gadus virens* and *Drepanopsetta platessoides*. With the exception of the cod these are all new British hosts. It is now known to occur in 17 British fishes but that number will be largely added to. Like *Derogenes varicus* it is essentially a parasite of Gadoid fishes, but to a more marked degree. It also occurs in somewhat larger numbers than that species.

It has been described by Odhner (1904, p. 351), Lebour (1908, pp. 56—57) and myself (1907, pp. 86—88 and 1909 *b*, pp. 20—21).

Species 2. *Hemiurus lühei* Odhner 1904.

This species was found only in the herring (*Clupea harengus*) to the extent of 25 %. It is described by Odhner (1904, p. 352), Looss (1908, p. 105) and myself (1907, pp. 85—95 and 1909 *b*, 21—22).

Sub-order GASTEROSTOMATA Odhner 1904.

Genus i. *Prosorhynchus* Odhner 1904.

Species 1. *Prosorhynchus aculeatus* Odhner 1904.

This species is a frequent parasite of the intestine of the conger and has been met with by several observers. It was confused with *Prosorhynchus crucibulum* (Rud.) and it was only first recognised as distinct from that species by Odhner. It occurs in moderate numbers and is apparently confined to the conger.

Except in young specimens it possesses a distinct yellowish-brown colour. The body is thick, but slightly flattened dorso-ventrally; of almost elliptical shape but pointed towards each end. The cuticle is entirely covered with regular scale-like spines.

The length of mature specimens is 1—2·5 mm. Egg-production begins at a length a little over 1 mm. The maximum breadth is at the middle of the body and is about half the length. The dimensions of an average full-grown specimen are $2 \times 0{\cdot}95$ mm. At the anterior end the

rhynchus (or rostellum) may be protruded like a small button, or retracted. In the latter case a shallow sucker-like depression is formed. The rhynchus is of simple, almost ovoid shape and its diameter is nearly twice its depth; it measures 0·27 × 0·15 mm. The mouth is situated little more than one quarter of the body length from the posterior end. The pharynx is flat and circular with a diameter of 0·14 mm. The intestine is a simple sac, extending forward from the mouth but not reaching the centre of the body. The excretory vesicle extends from the posterior end of the body as far forward as the level of the anterior testis, *i.e.* a short distance in front of the pharynx. It is a simple sac.

The testes are somewhat variable in position. They are of longitudinally oval shape and situated one on each side of the pharynx. The left testis is always in advance and its most usual position is on the level of the anterior border of the pharynx, while the right testis lies on the level of the posterior border of the pharynx. They vary about these positions, occasionally approximating the same level, but they are never absolutely symmetrical. Their dimensions are 0·26 × 0·20 mm. The cirrus-pouch is thick and of moderate length, extending forward to the posterior border of the left testis or to about a third of the body length from the posterior end. It thus extends a little in front of the pharynx, but occasionally just reaches the centre of the latter. It is invariably directed towards the left side of the body. An oval vesicula seminalis of medium size is situated outside the distal end of the cirrus-pouch and overlying it. It is connected with the pars prostatica by a narrower portion lying within the cirrus-pouch. The genital aperture is near the posterior end of the body.

The ovary lies on the right side almost exactly on the level of the middle of the body. It is thus a little further forward than the left testis. It is longitudinally oval in shape and not much smaller than the testes. The yolk-glands form a very symmetrical arc in the anterior part of the body. The transverse portion of this arc crosses the body about 0·4 mm. from the anterior end; the lateral portions extend back along each side to near the middle of the body, *i.e.* to near the level of the ovary. The follicles are large, and regularly disposed. Alternate follicles are situated on either side of the yolk-duct. The right yolk duct passes immediately in front of the ovary towards the centre of the body but the left duct has a longer course. It passes inwards in front of the left testis, crosses the intestine, then turns forwards to join the other at the level of the ovary. A receptaculum seminis is absent but Laurer's canal is present. The uterus is of great length and is thrown

into numerous convolutions. Beginning on the inner side of the ovary it passes backwards towards the right testis then turns and winds round the outer side of the ovary. Within the arc formed by the yolk-glands it makes a number of intertwining loops. It then passes round the left testis and so towards the genital sinus. This arrangement is almost peculiar to the species. The uterus is for the most part in front of the pharynx but it does not extend in front of the yolk-glands. The ova are numerous and of regular broad oval shape. They measure 0·026—0·031 mm. in length and 0·016—0·020 mm. in breadth, the average being 0·0285 × 0·0185 mm.

Species 2. *Prosorhynchus crucibulum* (Rud. 1819).

This is the second and larger of the two Gasterostomes, which are harboured by the conger. Like *P. aculeatus* it occurs in no other host. It was met with only half as frequently but it is probably quite as common as the other species.

Hitherto, although encountered by a number of observers, the species has not been correctly described. Originally described by Rudolphi as a Monostomum, it was redescribed by Molin (1858 and 1861) as a new species of Gasterostome. Rudolphi's description was recognised and amplified by Olsson (1876) who, however, curiously enough, failed to see that it was identical with Molin's species. With the latter he confused *P. squamatus* and *P. aculeatus*. Odhner (1904) disentangled this confusion and succeeded in defining the three species fairly aecurately. His diagnosis of *P. crucibulum*, however, based as it was on rather scanty material, is the least satisfactory of the three, and does not give an adequate idea of the form. Strictly speaking it is incorrect.

My material consists of about a dozen adult specimens from the Clyde and a large number of immature and a few adult specimens from the South coast. The length of adult individuals is 2—3·7 mm. Egg production does not occur under 2 mm. Molin gives the length as 4—6 mm. Odhner's size-limits (1·75—2·25 mm.) are, therefore, much too restricted.

The shape of the body is different from that of *P. aculeatus*; it is elongated, squarely cut at the anterior end and pointed posteriorly. It is slightly flattened and the greatest breadth (about $\frac{3}{8}$ths of the length) occurs near the middle. An average mature specimen measures 3·15 × 1·1 mm.

Large specimens have a rich yellowish-brown colour, due largely but not entirely to the numerous ova. Young ones are almost colourless. The whole body is covered with scale-like spines, most densely set anteriorly. The rhynchus is of very large size. In outline it is wedge-shaped, the apex of the wedge penetrating deeply into the body, and the base, forming the anterior end of the animal, being rounded and expanded. In certain cases the apex of the wedge is twisted, giving the rhynchus the "cornucopia" shape described by Molin. Frequently, especially in young specimens, the rhynchus forms a protruding button-like structure. Under normal circumstances the edge of the expanded anterior end is drawn out somewhat to form a projecting ridge, completely encircling the rhynchus. This is only well seen when the animal is alive. In a 3·15 mm. specimen the dimensions of the rhynchus are $0{\cdot}62 \times 0{\cdot}57$ mm. The histological structure closely resembles that of *Prosorhynchus squamatus* as described by Odhner.

The mouth is situated almost exactly in the centre of the body. The pharynx is circular and measures 0·21 mm. in diameter. The intestine extends forwards for a distance of about 0·3 mm. from the pharynx. It is an ovoid or globular sac, connected with the pharynx by a narrower portion. The excretory vesicle is comparatively short, extending forward about a third of the body length from the posterior end, *i.e.* to the level of the right testis.

The position of the genital glands is a feature on which no two descriptions agree. Molin places the testes symmetrically midway behind the pharynx and the ovary in front of the left testis. Olsson was able to see only two of the glands, one on each side of the pharynx and almost on the same level. In all probability the one on the right was the ovary. According to Odhner they have the same relative positions as in *P. squamatus*. I have found specimens corresponding with those described by Olsson and Odhner but never one in which the ovary was on the left side. Molin's drawing, however, was almost certainly made from the dorsal surface. The species, in respect of the genital glands, is by far the most variable which I have ever examined. No two specimens are absolutely alike and it is difficult on that account to indicate what may be considered the normal or typical structure. From my specimens it would appear that the condition more nearly approaches that in *P. aculeatus* than in *P. squamatus*, in so far as the testes are much more frequently placed across the body than one behind the other. In every case they are behind the pharynx, but nearer it than the posterior end of the body. In the majority they are placed one

towards each side of the body, with the left testis somewhat in advance of the right. They however may approach each other and even overlap, the left still being in advance. The right one, on the other hand, may be in advance, and from this overlapping position they may diverge till they come to lie obliquely tandem with the right in advance. In this position they are never widely separated and generally lie towards the right side of the body. The position of the ovary varies correspondingly. When the testes are in the first position described, it is usually situated immediately behind the pharynx, in the middle line. As the testes change in position it tends to move obliquely forwards to take up a limiting position at the right side of the pharynx.

It is impossible then to describe in a word the topography of the genital glands, but if that be attempted it were best summed up in: Ovary towards the right side, just behind the level of the pharynx; testes, oblique, a short distance behind the pharynx.

The testes are usually almost globular and have a diameter of 0·28 mm. The cirrus-pouch is short and stout and its extremity lies about a third of the body length from the posterior end. It usually lies towards the left side. Its internal structure is similar to that in *P. aculeatus*.

The diameter of the ovary is 0·25 mm. and it is thus little less than the testes. Just behind it lies a large shell-gland, with a Laurer's canal but no receptaculum seminis. The yolk-glands form a continuous arc across the body behind the rhynchus. The follicles are more irregular in size and disposition than those of *P. aculeatus*, and they are more massed together. The lateral follicles extend back to about the level of the blind end of the intestine, there being usually a slight inequality on the two sides so that those of the left reach a trifle further back than those on the right. The yolk-ducts are very long and pass down one on each side to unite behind the ovary.

The uterus is as a rule not so voluminous as in *P. aculeatus* and it is only in specimens over 3 mm. that it attains any great length. In smaller specimens it is confined very much to the left side and consists merely of an ascending limb from the ovary to the middle of the yolk-glands and thence a descending limb to the genital sinus. It increases in size by sending a loop down on the right side of the cirrus-pouch, but mainly by forming several convolutions in front of the pharynx, between it and the yolk-glands. The ova are very numerous and light yellow to dark brown in colour. They measure 0·026—0·030 mm. by 0·016—0·021 mm. the average being $0·029 \times 0·019$ mm. They are therefore of much the same size as those of *Prosorhynchus aculeatus*.

CESTODA.

As already remarked, Cestode parasites were rather uncommon, the only fish to harbour adult tapeworms being *Cottus scorpius* and *Gadus pollachius*. In 40 % of the former *Bothriocephalus bipunctatus* Zeder was found, and in 55 % of the latter *Abothrium rugosum* (Goeze) occurred. Both of these parasites are common and well-known forms and require no additional description. *B. bipunctatus* is an exceptionally frequent parasite of the turbot (*Bothus maximus*) and *Abothrium rugosum* is a typical Gadoid parasite.

Bothriocephalus scolices were found in the intestine of *Gobius ruthensparri* and *Labrus berggylta* but in very scanty numbers.

Scolex polymorphus Rud., which, in one or other of its various forms, is probably the commonest parasite of marine Teleostean fishes, was found in the alimentary canal of ten different fishes. It occurred most frequently in *Drepanopsetta platessoides, Conger conger* and *Labrus berggylta*, being present in at least 50 % of these fishes. Of the total number of fish examined at Millport 16 % were infected by this parasite. This figure is exceeded only by *Podocotyle atomon* (22 %).

This peculiar scolex is a composite form and includes the larvae of several species of *Calliobothrium* which become adult in the intestine of Elasmobranch fishes. A full description of it will be found in Zschokke (1889, pp. 251—259, Pl. VI, figs. 103—4).

A LIST OF THE FISHES EXAMINED, WITH THEIR PARASITES.

ACANTHOPTERYGII.

Scomber scombrus.

Pharyugora bacillaris (Molin).	Intestine.
Lecithocladium excisum (Rud.).	Stomach.
Scolex polymorphus Rud.	Intestine.

Cottus scorpius.

Podocotyle atomon (Rud.).	Intestine.
Stephanochasmus baccatus Nicoll.	Rectum.
Derogenes varicus (Müller).	Stomach.
Bothriocephalus bipunctatus Zeder.	Intestine.
Scolex polymorphus Rud.	Intestine.

Cottus bubalis.

Podocotyle atomon (Rud.)	Intestine.

ACANTHOPTERYGII (*cont.*).

Callionymus lyra.

Lebouria varia Nicoll.	Stomach and intestine.
Zoogonoides viviparus (Olss.).	Rectum and intestine.
Scolex polymorphus Rud.	Intestine.

Trigla pini.

Lecithaster gibbosus (Rud.).	Intestine.
Scolex polymorphus Rud.	Intestine.

Gobius ruthensparri.

Lecithaster gibbosus (Rud.).	Intestine.
Bothriocephalus scolex.	Intestine.

Pholis gunnellus.

Podocotyle atomon (Rud.).	Intestine.
Scolex polymorphus Rud.	Intestine.

PHARYNGOGNATHI.

Labrus berggylta.

Peracreadium genu (Rud.).	Rectum.
Peracreadium commune (Olss.).	Rectum.
Lebouria alacris (Lss.).	Intestine.
Helicometra pulchella (Rud.).	Rectum and intestine.
Lecithaster gibbosus (Rud.).	Intestine.
Hemiurus communis Odhn.	Intestine.
Scolex polymorphus Rud.	Rectum.
Bothriocephalus scolex.	Rectum.

ANACANTHINI.

Gadus minutus.

Derogenes varicus (Müller).	Stomach.
Hemiurus communis Odhn.	Stomach.
Scolex polymorphus Rud.	Coeca.

Gadus callarias.

Stephanochasmus pristis (Deslongch).	Coeca.
Derogenes varicus (Müller).	Stomach.
Hemiurus communis Odhn.	Stomach.
Scolex polymorphus Rud.	Gall bladder.

Gadus virens.

Podocotyle atomon (Rud.).	Coeca and intestine.
Lepidapedon rachiaenm (Cobbold).	Intestine.
Derogenes varicus (Müller).	Stomach.
Hemiurus communis Odhn.	Stomach.

Gadus merlangus.

Lecithaster gibbosus (Rud.).	Intestine.
Derogenes varicus (Müller).	Stomach.

ANACANTHINI (*cont.*).

Gadus pollachius.

Podocotyle atomon (Rud.).	Intestine.
Lepidapedon rachiaeum (Cobbold).	Intestine.
Derogenes varicus (Müller).	Stomach.
Hemiurus communis Odhn.	Stomach.
Abothrium rugosum Goeze.	Coeca and intestine.
Scolex polymorphus Rud.	Intestine.

Pleuronectes limanda.

Podocotyle atomon (Rud.).	Intestine.
Stephanochasmus baccatus Nicoll (larva).	Muscles.
Leioderma furcigerum (Olss.).	Intestine.
Zoogonoides viviparus (Olss.)	Rectum and intestine.

Pleuronectes microcephalus.

Leioderma cluthense Nicoll.	Coeca and intestine.
Zoogonoides viviparus (Olss.).	Rectum and intestine.

Pleuronectes platessa.

Lebouria varia Nicoll.	Intestine.
Podocotyle atomon (Rud.).	Intestine.
Zoogonoides viviparus (Olss.).	Rectum and intestine.
Derogenes varicus (Müller).	Stomach.
Cryptocotyle concava (Crepl.) (larva).	Muscles.

Drepanopsetta platessoides.

Lecithaster gibbosus (Rud.).	Intestine.
Hemiurus communis Odhn.	Stomach.
Scolex polymorphus Rud.	Intestine.

PHYSOSTOMI.

Clupea harengus.

Hemiurus lühei Odhn.	Stomach.

Conger conger.

Helicometra pulchella (Rud.).	Rectum.
Lecithochirium rufoviride (Rud.).	Stomach.
Prosorhynchus aculeatus Odhn.	Intestine.
Prosorhynchus crucibulum (Rud.).	Intestine.
Scolex polymorphus Rud.	Rectum.

The following fishes contained no platyhelminth parasites:

Agonus cataphractus, *Trigla gurnardus*, *Gasterosteus aculeatus* (var. *gymnurus*), *Gastraea spinachia*, *Ctenolabrus rupestris*, *Pleuronectes cynoglossus*, *Scophthalmus unimaculatus*, *Anguilla vulgaris*, *Syngnathus acus*, *Nerophis lumbriciformis.*

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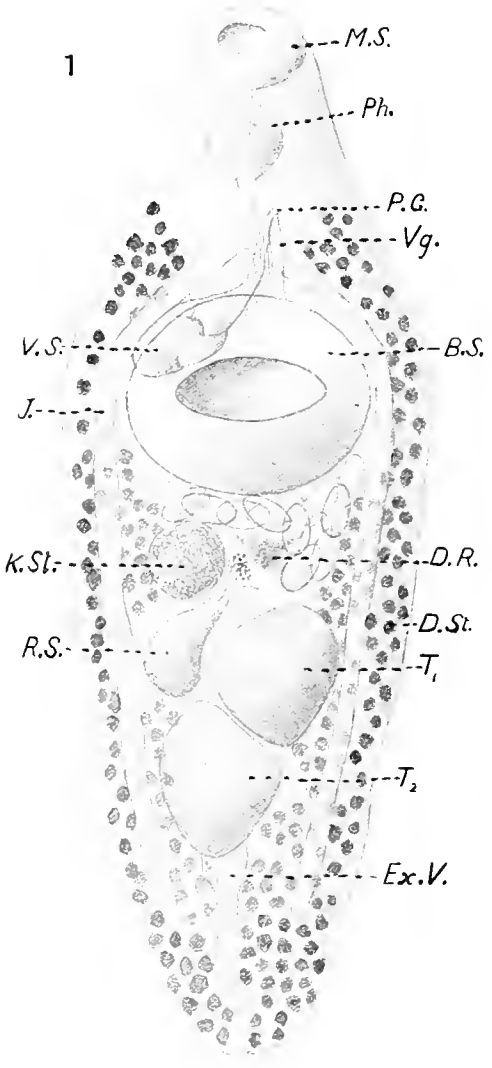

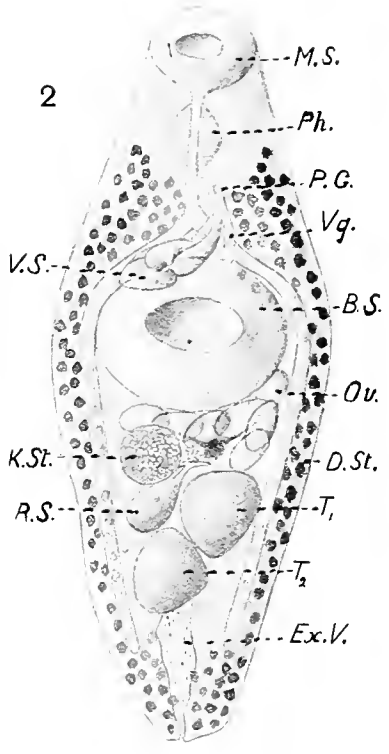

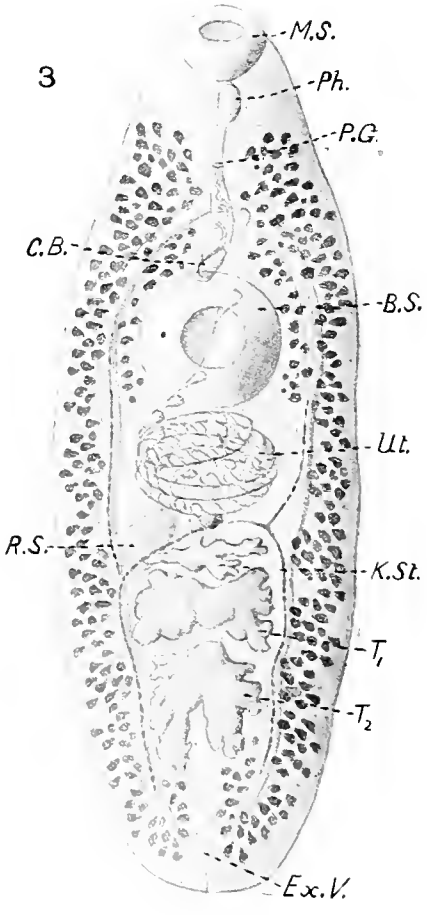

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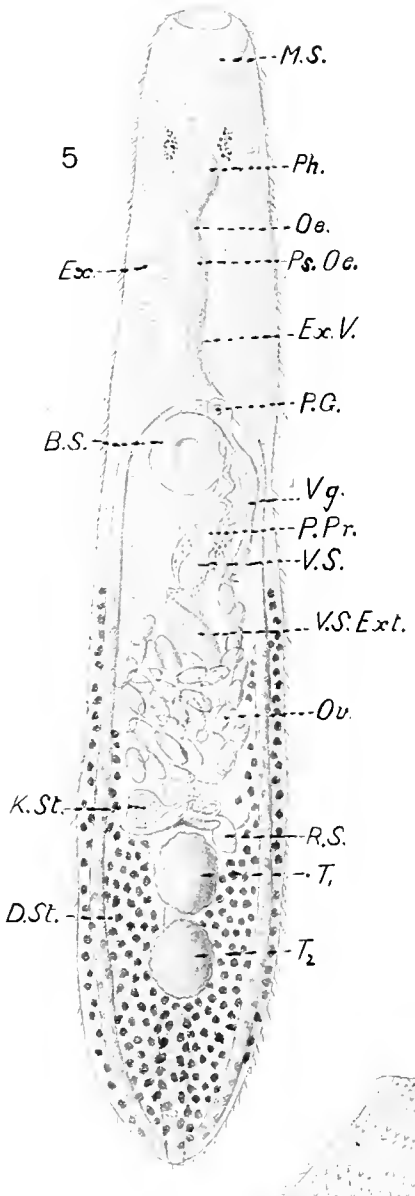
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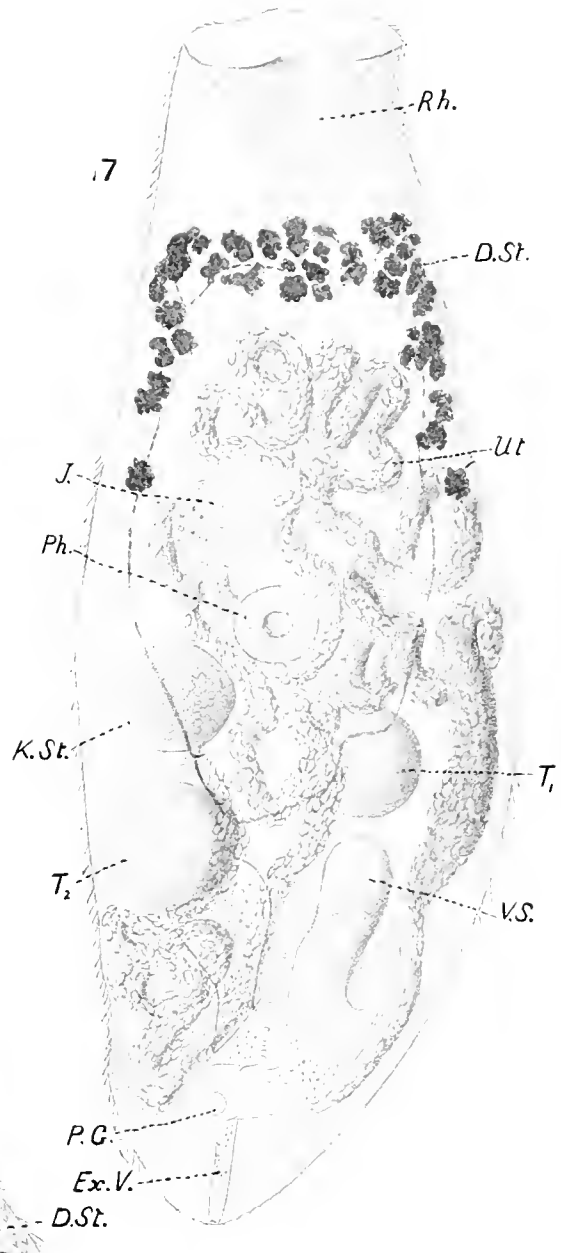
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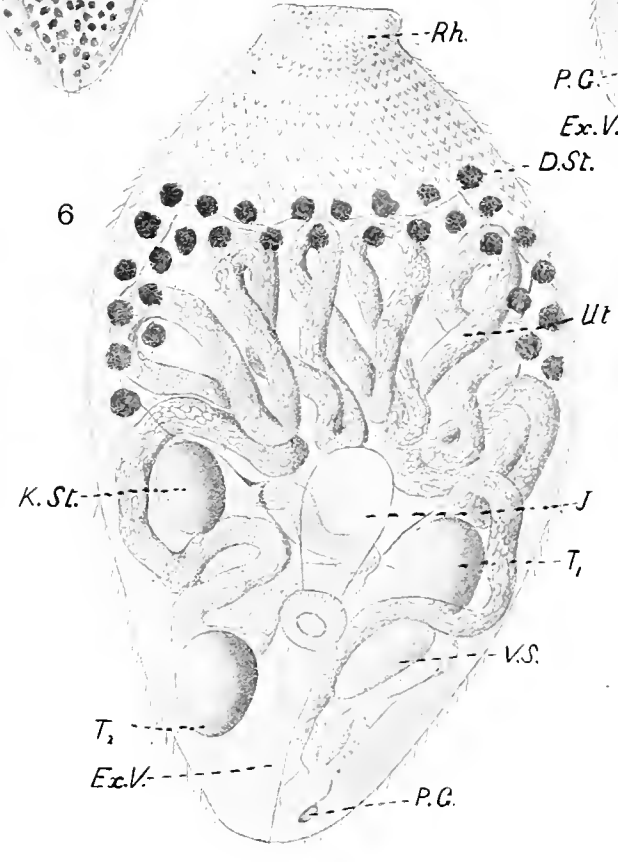
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EXPLANATION OF PLATE XXIX.

The following letters apply to all the figures:—

B.S.	Ventral sucker.	*Ph.*	Pharynx.
C.B.	Cirrus pouch.	*P.Ph.*	Prepharynx.
D.E.	Ductus ejaculatorius.	*Ps.Oe.*	Pseudo-oesophagus.
D.St.	Yolk glands.	*P.Pr.*	Pars prostatica.
Ex.V.	Excretory vesicle.	*Pr.*	Prostate glands.
Ex.	Excretory tubules.	*Rh.*	Rhynchus.
J.	Intestinal diverticula.	*R.S.*	Receptaculum seminis.
K.St.	Ovary.	*S.D.*	Shell gland.
L.C.	Laurer's canal.	T_1, T_2.	Testes.
M.S.	Oral sucker.	*Ut.*	Uterus.
Oe.	Oesophagus.	*Vg.*	Vagina.
Ov.	Ova.	*V.S.*	Vesicula seminalis.
P.G.	Genital aperture.	*V.S.ext.*	Vesicula seminalis externa.

Fig. 1. *Lebouria varia*, n.sp. Ventral view. ×80.
Fig. 2. *Lebouria alacris* (Lss.). Ventral view. ×80.
Fig. 3. *Helicometra pulchella* (Rud.). Ventral view. ×50.
Fig. 4. ,, ,, ,, Ovum. ×275.
Fig. 5. *Pharyngora bacillaris* (Molin). Ventral view. ×66.
Fig. 6. *Prosorhynchus aculeatus* Odhner. Ventral view. ×45.
Fig. 7. *Prosorhynchus crucibulum* (Rud.). Ventral view. ×45.

THE DEVELOPMENT OF TRYPANOSOMA LEWISI OUTSIDE THE VERTEBRATE HOST[1].

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(*From the Quick Laboratory, Cambridge.*)

With 21 Diagrams.

CONTENTS.

1. *Introduction.*

Since the publication of Prowazek's paper (1905) on the development of *Trypanosoma lewisi* in the rat louse (*Haematopinus spinulosus*) our knowledge about this question has not been increased very much. When we consider the papers of Prowazek, Baldrey (1909), Rodenwaldt (1909) and Breinl and Hindle (1910) we may state the following facts:

The trypanosomes may develop in the louse under certain circumstances not exactly known. They change their form, become first

[1] A short account of these investigations was given at the meeting of the Cambridge Philosophical Society of June 6, 1910.

elongated crithidia-like forms, with the nuclear apparatus situated in the hind end; then they shorten up and take the form of short oval crithidiae[1] (with an undulating membrane) or herpetomonads (without an undulating membrane), which divide actively, forming rosettes. Round forms may be found also, but the authors could not make clear their significance or the way in which they are formed. Some of the larger herpetomonads, with short flagella or aflagellar and with an indistinct blepharoplast, are considered to be ookinetes arising from a previous conjugation. Some authors (Prowazek, Baldrey, Rodenwaldt) assert that they have seen a heterogamic sexual process, but their proofs for this statement are far from convincing. Apart from these forms, Prowazek described reduction of the blepharoplast and the nucleus, male and female forms, etc.

Minchin and Thomson (1910) have pointed out that there is a distant transmission of *T. lewisi* from rat to rat, which is performed by the rat flea (*Ceratophyllus fasciatus*), and so they think that this flea is the real host for the trypanosome, a statement which confirms Swingle's assertion (1907), that there is development of this flagellate in the flea's gut.

As is well known, all these papers have been severely criticised by Patton and Strickland (1908), who assert that the *Crithidiae* found in the louse and the flea are natural flagellates and have nothing to do with *T. lewisi.* This argument must be specially considered in connection with the supposed development of *T. lewisi* in the rat flea, because Swingle (1907), Balfour (1906) and MacKinnon (1909) have described various herpetomonads and *Crithidiae* in *Pulex cleopatrae* (Balfour's *Herpetomonas*), *Ctenophthalmus agyrtes* (*Herpetomonas ctenophthalmi* MacKinnon) and *Hystrichopsylla talpae* (*Crithidia hystrichopsyllae* MacKinnon). After the description of the forms we observed, we shall have occasion to discuss this question and the influence these observations have on the interpretation of our work.

The object of our experiments was:

(1) To trace out the development of *T. lewisi* (if there were any) in the rat flea (*Ceratophyllus fasciatus*).

(2) To compare this development with that in the rat louse, in some other Arthropods and in artificial cultures, to make sure if the development in the invertebrate host is a specific life-cycle or only a sort of natural culture.

[1] The word is used in a descriptive sense throughout this paper when it is not italicised.

2. *Material and Methods.*

The fleas used for the study of the supposed life-cycle were hatched out from larvae preserved in a flea-breeding apparatus. This culture existed since 24th November, 1908. The fleas were fed on uninfected rats. The lice were directly taken from the rats because it is impossible to keep them alive without constantly feeding them on a rat. For the study of the living flagellate the intestines (of flea or louse) were taken out, without rupturing them, and were put between a slide and cover-slip. Sometimes such preparations were kept for 24 hours in an incubator at 28° C.; but the death of the parasites prevented generally a more prolonged observation. In order to make stained preparations, different parts of the gut (of flea or louse) were teased in a drop of 0·6% salt solution and smeared out. The preparations were then put in absolute alcohol before they were dried completely and stained with Giemsa's solution. To produce a satisfactory staining it was generally necessary to stain for 12 hours. Beside this method other preparations were fixed, without drying, in corrosive alcohol and subsequently stained with Heidenhain's iron hematoxylin (Breinl's modification). Both methods gave satisfactory results which did not differ at all from one another. We found the old method of fixing and staining a very good one, not deserving the severe criticisms to which it has been subjected of late (cf. Swellengrebel, 1910).

3. *The development of T. lewisi in the rat flea (Ceratophyllus fasciatus).*

To study this point 83 fleas out of the uninfected laboratory culture were allowed to feed for 12 hours on an infected rat, which had been infected ten days before and showed trypanosomes in the last stage of the period of division. After feeding they were taken off and preserved in a gauze bag at 13—16° C., where they were allowed to feed each third day on a non-infected rat. Each day for 18 days 4—6 (sometimes more) fleas were dissected and preparations were made of the contents of the different parts of the gut (midgut, hindgut, rectum). The following table (I) shows the rate of infection for each day.

We see that 44·6% of these fleas showed stages of development and even when excluding the first two days (during which the morphological alteration was a very slight one) the rate of infection was still 36·9%.

TABLE I. (*Series F.*)

Day after feeding	Number of infected fleas	Number of non-infected fleas	Day after feeding	Number of infected fleas	Number of non-infected fleas
½	3	0	10	2	2
1	2	0	11	1	2
2	5	0	12	1	2
3	2	3	13	1	1
4	3	3	14	1	3
5	2	12	15	2	2
6	2	5	16	1	3
7	3	1	18	1	1
8	1	3			
9	4	3	Total	37	46

Because the possibility existed that the flagellates we found in the fleas of Series F were natural forms (in accordance with Patton's views), we started a control series with 60 fleas out of the same culture. These fleas were allowed to feed for 12 hours on an uninfected rat and were treated afterwards in the same way as the fleas of Series F. Table II shows the results of this experiment.

TABLE II. (*Series G.*)

Day after feeding	Number of infected fleas	Number of non-infected fleas	Day after feeding	Number of infected fleas	Number of non-infected fleas
½	0	4	8	0	4
1	0	2	9	1	2
2	0	4	10	0	12
3	1	3	11	0	7
4	0	5	12	0	2
5	0	4	13	0	2
6	0	4			
7	0	3	Total	2	58

Here we see that 3·3% of the control fleas were infected, or (if we do not take into consideration the first two days) 4%. This infection of the control fleas was probably due to an infected wild rat having broken into the flea box. But even if we consider the flagellates in the control fleas to be natural forms which have no connection with *T. lewisi*, we may safely conclude that the majority of the forms found in the fleas of Series F were developmental forms of *T. lewisi* because the rate of infection was much larger in Series F (36·9%) than in Series G (4%). This difference becomes more striking when we consider that generally a flea of Series F was only considered infected

when flagellates were to be found in the freshly dissected unstained gut, whereas in Series G no flea was considered to be free from flagellates unless the observation *in vivo* was confirmed by the study of stained preparations.

This latter precaution is necessary because there were always very few flagellates to be found in the fleas of Series G so that they might easily have been overlooked during the observation of the fresh gut. The infection of the two fleas of Series G was only seen in stained preparations.

We will now proceed to describe the various forms found on each day in the fleas of Series F.

Twelve hours after feeding. During the first hours of their stay in the midgut of the flea the trypanosomes undergo many changes which affect the structure of the nucleus and blepharoplast, which have nothing to do with the subsequent development, and resemble the changes undergone by trypanosomes preserved for 7—11 days in the refrigerator (hypertrophy of the blepharoplast combined with the formation of chromidia, asymmetric segmentation of the nucleus, etc.). One of us (N. H. S. in a paper about to be published in *Parasitology*) will deal with the different forms of degeneration exhibited by *T. lewisi* and will show how many of the figures interpreted by

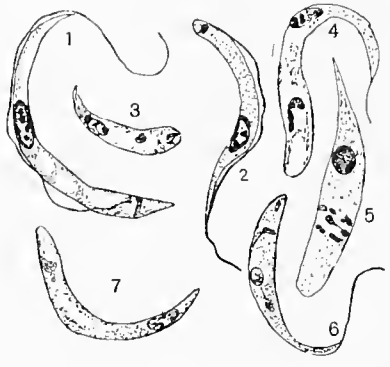

Diagram I.

Fig. 1. Ordinary trypanosome. *G.* (dry fixation, Giemsa stain).
Fig. 2. The same as Fig. 1. *I. H.* (wet fixation, Heidenhain stain).
Fig. 3. Small trypanosome. Nucleus throwing out chromidia. *G.*
Fig. 4. Trypanosome with blepharoplast throwing out chromidia. *G.*
Fig. 5. Trypanosome with fragmented blepharoplast (resembling mitotic division of the blepharoplast, described by Prowazek). *G.*
Fig. 6. Asymmetric nuclear division. *G.*
Fig. 7. Hypertrophied blepharoplast throwing out chromidia. *G.*

some authors as a reduction of the nucleus and blepharoplast are probably nothing but such degenerating forms; so we will not describe them here, but merely give some characteristic types showing fragmentation of the nucleus (Diagram I, Figs. 3, 6), hypertrophy and fragmentation of the blepharoplast (Figs. 4, 5, 7). Other forms are identical to those found in the blood of the rat, with an alveolar protoplasm and a nucleus with one or two central chromatic granules (Fig. 1). The "never dried" preparations stained after Heidenhain's method exhibit absolutely the same structure (Fig. 2).

The trypanosomes become very soon agglomerated when the gut wall is ruptured; this phenomenon might be falsely interpreted as conjugation, because the flagellates often agglomerate in pairs.

Twenty-four hours. The greater part of the trypanosomes in the midgut exhibit the following changes in their structure. The blepharoplast leaves its position at the posterior end of the flagellate and wanders in the direction of the nucleus (Diagram II, Fig. 1). The

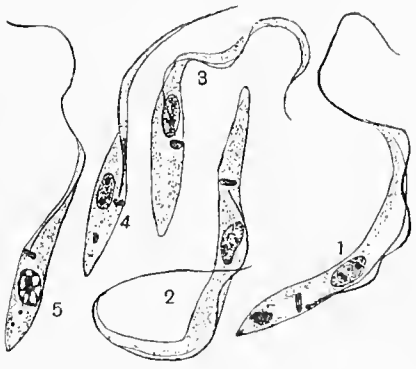

Diagram II.

Figs. 1—5. Different stages of the wandering of the nucleus and the blepharoplast. *G.*

nucleus on the other hand moves toward the hind-end of the cell (Fig. 2). So the distance between the nucleus and blepharoplast diminishes gradually (Fig. 3). Finally, the two pass each other (Fig. 4), and the blepharoplast comes to lie anterior to the nucleus, the trypanosome having now a *Crithidia* facies (Fig. 5).

These changes bear a strong resemblance to those described by Chagas (1909) in *Schizotrypanum cruzi* in the gut of *Conorrhinus*, but we never saw any sign of fusion of the nucleus and the blepharoplast. The structure of the nucleus is the same as in the ordinary trypanosomes, the blepharoplast often appears to be composed of distinctly

chromatic and achromatic substance (Figs. 1, 3, and 5); the basal granule of the flagellum is sometimes well seen (Fig. 1).

Trypanosomes taken from the flea's gut, immediately after feeding, and kept in the incubator at 28° C. between the slide and cover-slip, showed also this transformation into crithidiae (Diagram III, Fig. 1),

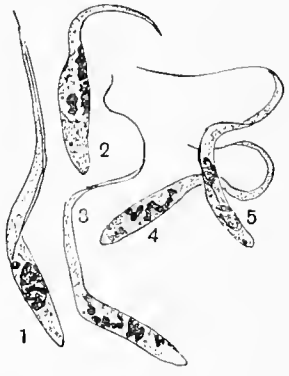

Diagram III.

Fig. 1. Crithidia. *G.*

Fig. 2. Chromidia arranged in a filament near the nucleus; blepharoplast absent. *G.*

Figs. 3 and 4. Chromidia arranged in a twisted filament; nucleus and blepharoplast absent. *G.*

Fig. 5. Two nuclei are present; the blepharoplast situated between them. *G.*

but besides many degenerative changes occur which are not observable in the forms taken directly from the gut. The nucleus was often divided, a change which was never observed in the normal forms after 24 hours. The blepharoplast was situated between the two nuclei (Fig. 5). Often the nucleus was completely degenerated and changed into a filament of chromidia (Figs. 2, 3, and 4), the blepharoplast having quite disappeared. These forms are very much alike to the "male" forms described by Prowazek and Baldrey in the louse, and to some of the forms found in the refrigerator by one of us (N. H. S.). The end of the degeneration was the complete disappearance of the protoplasm, only the flagellum remaining.

In the living preparations the normal forms of 24 hours, as well as the forms preserved in the incubator, show another change in the general size, the hind-end being more or less thickened, so that the whole cell has the shape of a club.

Second day. The number of the crithidiae is larger at this time, but otherwise the same forms may be found as during the previous stage (Diagram IV). Development still occurs in the midgut, although sometimes active flagellates may be found in the pyloric region.

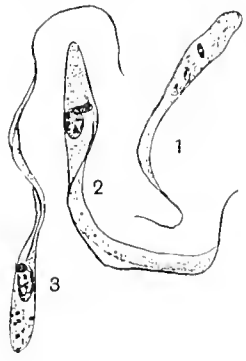

Diagram IV.

Figs. 1—3. Different stages of the wanderiug of the nucleus and the blepharoplast. *G.*

Third day. The crithidiae are now abundant in the midgut, also some pre-crithidial stages are yet to be found (Diagram V, Fig. 2).

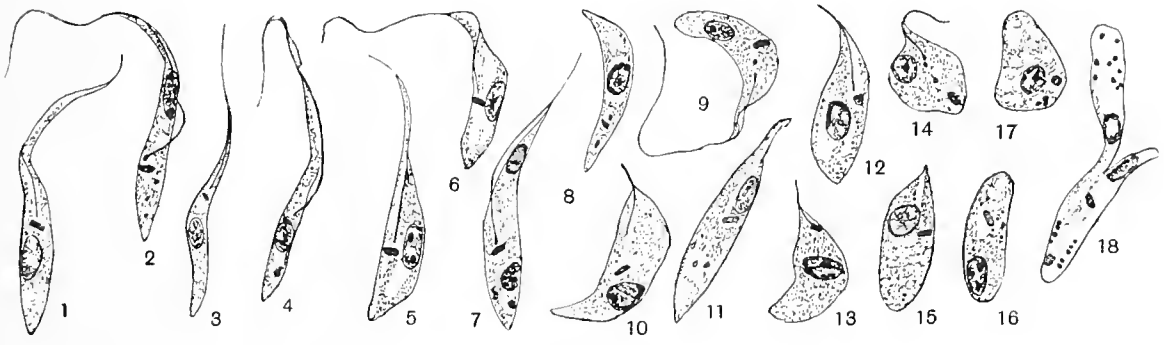

Diagram V.

Figs. 1—2. Transformation of a trypanosome into a crithidia. *G.*
Figs. 3—4. Slender crithidiae. *G.*
Figs. 5, 6, 9. Stout crithidiae (formation of large oval forms). *G.*
Fig. 7. Stout crithidia; blepharoplast between the two nuclei. *G.*
Fig. 8. Stout crithidia, flagellum unstained, blepharoplast and nucleus close together. *G.*
Figs. 10—12. Large oval forms, Figs. 10 and 11, *G*, Fig. 12, *I. H.*
Figs. 13—16. Large oval forms being transformed into round forms. *G.*
Fig. 17. Round form. *G.*
Fig. 18. Pseudo copulation. *G.*

Among the crithidiae two types may be distinguished:

(*a*) Club-shaped forms (Figs. 1, 6, and 9) often with a more or less reduced flagellum (Figs. 5, 7, and 8).

(*b*) Slender crithidiae with a well-developed flagellum (Figs. 3 and 4).

Both types are connected by intermediate forms. One might be tempted to consider this differentiation as a sexual one, but we never

saw any sign of conjugation, and moreover the broad forms go on with their development in a very different way, so we are inclined to believe that the thin forms are incipient broad ones.

Fig. 7 shows again a form such as that seen above (Diagram III, Fig. 5) with two nuclei and the blepharoplast between the two, but these forms are uncommon and we mention them as a curiosity. Another one is figured in Fig. 8. The flagellum is absent (or not well stained), the blepharoplast seems to be fused with the nucleus, but probably it is a mere supposition. In point of fact, the blepharoplast never disappears during the whole process of development. We mention this form because the resemblance with Prowazek's "ookinete" is so striking.

The broad, club-shaped forms are transformed into the "large oval forms" (Figs. 10—12), the flagellum shortening up and the undulating membrane disappearing. The blepharoplast is situated beside or in front of the nucleus. In this stage of development the flagellates generally pass into the hindgut, but further development may take place in the midgut. The large oval forms become gradually shorter and broader (Figs. 13—16), the flagellum is very short and often difficult to stain, so that it is only to be detected as a bright line arising from the blepharoplast (Fig. 15). Sometimes no trace of a flagellum is to be seen (Fig. 16) but we doubt if it has really disappeared and are inclined to believe that it stains only with difficulty at this stage.

These forms pass into the round forms (Fig. 17) which may be found already in the midgut, but which are abundant only in the hindgut and the rectum.

The nuclear structure of all these forms is not essentially different from the normal structure. Sometimes the central chromatic mass (karyosome) disappears; which is to be seen in the preparations stained after Giemsa and Heidenhain (Figs. 10, 12, and 15). The basal granule of the flagellum is often well marked (Figs. 2 and 9) but if the flagellum is not well stained the basal granule is invisible.

Curious effects of agglomeration suggesting conjugation were sometimes observed (Fig. 18).

Fourth day. No flagellates are to be seen in the midgut. In the pyloric region of the hindgut, free and attached forms are to be observed. The latter are attached to the gut wall or to each other, forming rosettes; they are broad without apparent flagellum or undulating membrane. Apart from these forms the rosettes contain active club-shaped flagellates and round forms (cf. Diagram VIII).

Some of them are figured in Diagram VI, Fig. 2, a large oval form shows a double basal granule of the flagellum probably marking a subsequent division.

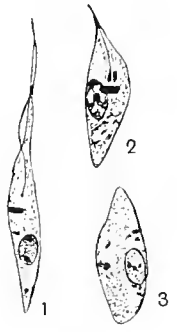

Diagram VI.

Fig. 1. Crithidia. *G.* Fig. 2. Large oval form. *G.* Fig. 3. Round form. *G.*

Fifth day. The fleas dissected on this day showed only very few round forms in the rectum (Diagram VII) probably they were washed out of the hindgut. Some of these forms showed division. The flagellum was only to be seen as a bright line within the cell.

Diagram VII.

Fig. 1. Dividing round form. *G.* Fig. 2. Non-dividing round form. *G.*

Sixth day. The same stages were to be seen as on the fourth day (Diagram VIII). The diagram shows two rosettes, one stained with Giemsa, the other with Heidenhain; Fig. 2 shows a large oval form (*a*) a slender crithidia, (*b*) thick crithidiae, (*c*) and a dividing round form, (*d*) in one rosette. The large oval form of this rosette has yet an external flagellum. Fig. 1 shows a rosette of large oval forms and a dividing round one. One of the large oval forms is dividing but this is rather uncommon, because the large oval forms generally become rounded up before the division begins. We do not exactly know how these rosettes of large oval and round forms originate; we do not think

it probable that they are mainly produced by division because active reproduction begins only after the formation of the rosettes. We suppose that these rosettes are formed by the apparent tendency of the large oval forms to adhere to anything, they may adhere to the gut-wall but also to one another. This phenomenon is quite different from the ordinary agglomeration. Some of the broad forms described by Rodenwaldt as "ookinetes" resemble our large oval forms.

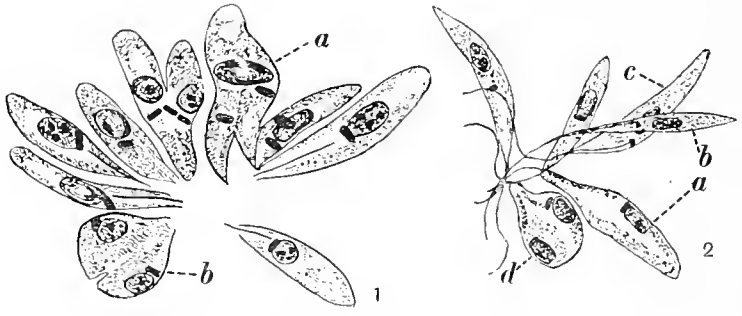

Diagram VIII.

Fig. 1. Rosette of large oval and round forms. *G.*
Fig. 2. Rosette of large oval and round forms, slender and thick crithidiae. *I. H.*

Seventh day. On the seventh day we found the number of round forms to be larger than on the previous days. The round forms arising from the large oval forms at this period are generally larger than those found later which arise from the small oval forms (see below). They generally possess a large nucleus (Diagram IX) with a well-marked

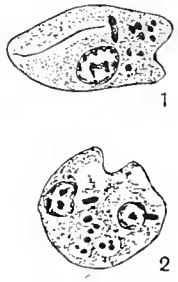

Diagram IX.

Figs. 1 and 2. Dividing and non-dividing round forms. *G.*

karyosome. This structure is dividing in Fig. 1, probably previous to the nuclear division, although the division of the karyosome is not necessarily followed by nuclear division. In Fig. 1 the blepharoplast is also dividing, the achromatic substance becoming elongated with the

two chromatic granules at both ends. In Fig. 2 the blepharoplast is divided and one of the nuclei shows undoubted signs of a renewed division, being elongated and more or less constricted in the middle.

The subsequent stages of nuclear division will be described below. A general peculiarity of the round forms is the occurrence of darkly staining granules scattered in the protoplasm. A flagellum may be present or absent, and if absent there may be no sign of any basal granule.

Eighth day and afterwards. Whereas the developmental stages of the previous days showed a more or less marked progression in relation to each other, this is no longer the case with the stages of the eighth and subsequent days. Although a great variety of forms are produced, there is no further progression to be observed and the forms found during these days are all the same, therefore we describe and figure them together.

In the unstained gut many oval inactive and active flagellates and round forms are adherent to the pyloric region and to the rectum. Beside these small active flagellates with a well-marked undulating membrane active oval forms and free round forms are to be seen in hindgut and rectum. There is no well-marked difference between the contents of hindgut and rectum; all the different types may be

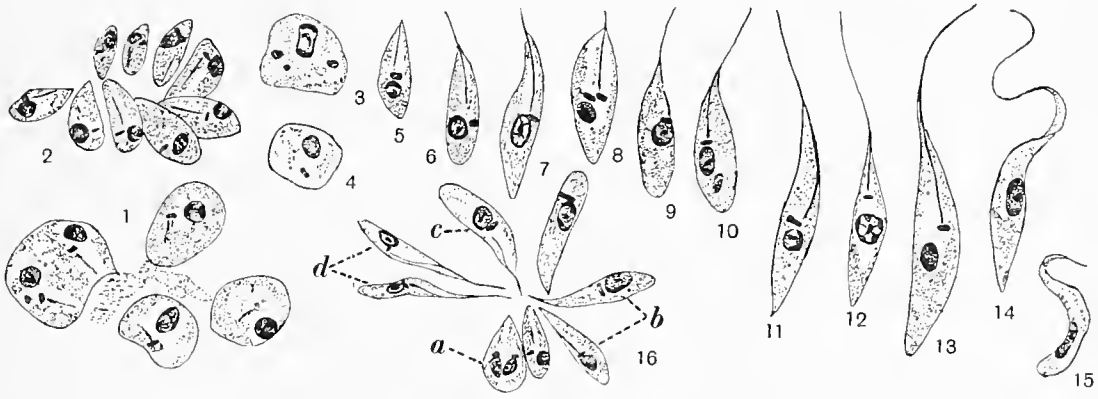

Diagram X. I. H.

Fig. 1. Rosette of round forms.
Fig. 2. Rosette of small oval forms.
Fig. 3. Round form with dividing nucleus.
Fig. 4. Small round form.
Figs. 5—12. Transformation of small oval forms into crithidiae.
Fig. 13. Large crithidia.
Figs. 14—15. Small trypanosomes.
Fig. 16. Rosette composed of small oval forms, crithidiae and intermediate forms.

found in both parts of the intestinal tract, although sometimes one or the other is favoured with the greatest amount of parasites.

The rosettes found in the pyloric region are no longer composed of large oval forms, but of round and small oval forms. Diagram XI, Fig. 2 shows a rosette of round forms without any external flagellum, dividing actively. The components of the rosette are united by a substance staining pink with Giemsa.

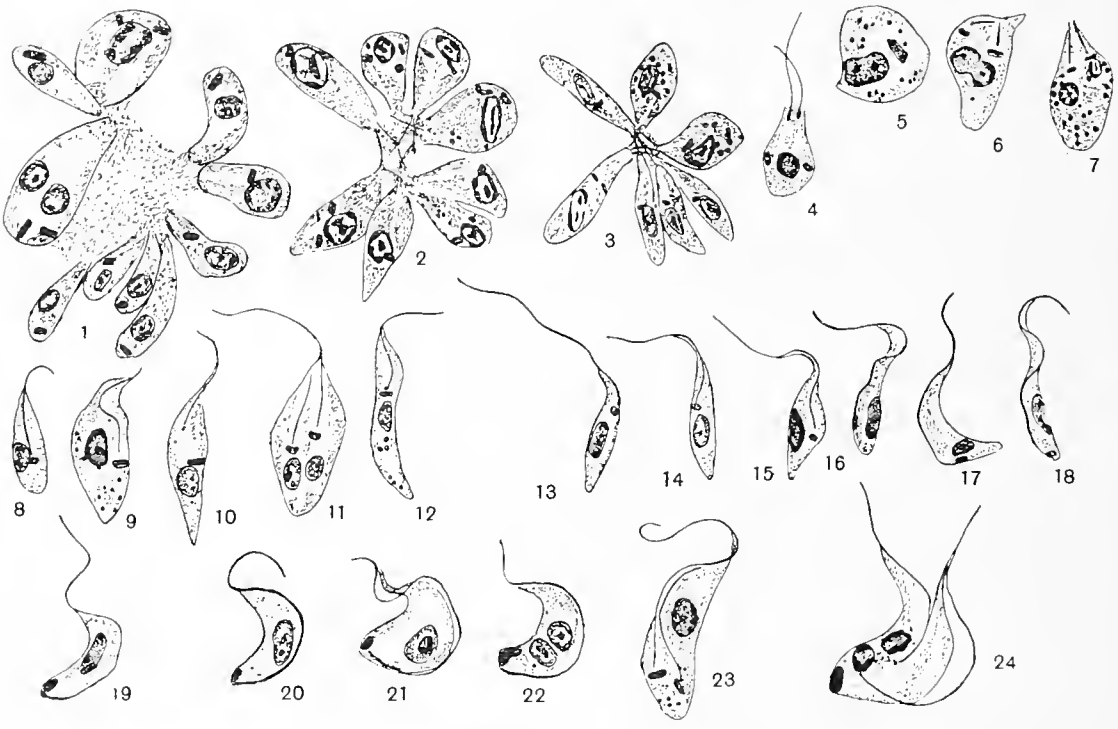

Diagram XI. G.

Figs. 1—3. Different forms of rosettes.
Figs. 4—7. Round forms.
Figs. 8—9. Small oval forms.
Figs. 10—12. Crithidiae.
Figs. 13—17. Intermediate forms between crithidiae and small trypanosomes.
Figs. 18—24. Small trypanosomes.

This intercellular substance is often found in the rosettes. Prowazek referred to it when describing the forms from the louse. Sometimes this pink matter is absolutely structureless (in Giemsa and Heidenhain preparations). Perhaps in these cases it is to be considered as a product of the periplast. In other cases the substance has a distinct reticular structure, and then it seems to be composed of the more or

less developed flagella. This is particularly clear in the Heidenhain preparations (Diagram VIII, Fig. 2).

Diagram XI, Figs. 1 and 3 show a rosette with round and small oval forms. By continuous division rosettes are produced which are wholly composed of small oval forms (Diagram X, Fig. 2).

The round forms, which may be found dividing when occurring singly or in rosettes, show distinctly the different stages of nuclear division (Diagram XI). Fig. 5 shows an elongated nucleus, slightly constricted in the middle with divided karyosome, the rest of the chromatin being situated in the periphery. In Fig. 6 the constriction is better marked, the peripheral chromatin has been retracted to the poles of the dividing nucleus. Fig. 5 shows also an interesting stage of the division of the blepharoplast. The chromatic portions at the two ends of the dividing organella are composed of a chromatic granule and a chromatic polar plate, which would suggest that the division of the blepharoplast is proceeding in the same way as the nucleus, the polar plate corresponding to the peripheral chromatin and the chromatic granule to the karyosome (centriole). However this may be, we saw such a figure only once, and we have no right to deduce any far-reaching conclusions from a single figure. The round forms contain many dark-staining granules. Generally they have no free flagellum but exceptions to this rule may be observed (Fig. 4).

In the wet-fixed Heidenhain preparations the structure of the nucleus is the same as in the Giemsa preparations (Diagram X, Figs. 1, 3 and 4). A karyosome is generally present but sometimes only peripheral chromatin is to be seen (Figs. 1 and 4) as is also the case in the Giemsa preparations (Diagram XI, Fig. 4). The structure of the blepharoplast is often as clear as after staining by Giemsa, but the nuclear division is not so distinct, owing to the shrinkage of the nucleus which is often considerable (Fig. 3). As in Giemsa preparations a pale-staining substance may be seen uniting the round forms in a rosette (Fig. 1).

The small oval forms arising from the division of the round forms have their blepharoplasts beside or in front of the nuclei (Diagram X, Fig. 2). At first the flagellum is only internal but grows out very soon (Figs. 5—6). As in the large oval and round forms there is often no flagellum to be seen in the small oval flagellates. We seriously doubt, however, whether the flagellum ever disappears, because none of the different types lacks a flagellum constantly; it is always to be found in a certain percentage of the different types. It seems probable that

the flagellum becomes achromatic under certain conditions, but that it is always present as an intercellular flagellum.

All the different types to be described here may show signs of division, but the multiplication in this last stage of development results mainly from the division of round and small oval forms.

The small oval forms become larger (Diagram X, Figs. 5—7, Diagram XI, Fig. 8) and may divide again (Diagram X, Fig. 8, Diagram XI, Fig. 9). When doing this they may be rounded up again, forming small round forms (Diagram X, Fig. 4). At last they are transformed into well-marked Crithidiae, the flagellum growing out (Diagram X, Figs. 9—12, Diagram XI, Figs. 10—12). A slender and a thick type (Diagram X, Fig. 13 and Diagram XI, Fig. 12) may be distinguished united by intermediate forms (Diagram X, Figs. 11, 12, Diagram XI, Fig. 10). These Crithidiae may divide also (Diagram XI, Fig. 11). One might think these forms to be sexually differentiated, but when looking at the variation in size of the Crithidiae found in cultures (cf. Diagram XII, Figs. 1—3) this dimorphism seems to be of no essential significance.

The slender Crithidiae undergo further changes (Diagram XI). The flagellum becomes longer and the undulating membrane more developed. At the same time the protoplasm, which stained dark blue, takes a paler blue colour. The development of the undulating membrane is due to the fact that the blepharoplast leaves its position anterior to the nucleus (Figs. 13, 14), comes first beside the nucleus (Figs. 16—17) and finally takes a position at the extreme posterior end of the cell (Fig. 18). The flagellate has now recovered the aspect of a perfect though small trypanosome, which is however very different from *T. lewisi* found in the blood not only in size but also in structure.

The protoplasm is stained pale blue with Giemsa. The blepharoplast, with its characteristic position already mentioned, is generally very large after Giemsa staining; in the Heidenhain preparations it is much smaller because in this case only the chromatic part is stained (Diagram X, Fig. 15). The basal granule is generally very distinct (Diagram XI, Fig. 19). The nucleus is sometimes round (especially previous to division) but it is often much elongated. Sometimes a karyosome is well marked, but often there are several chromatic granules to be found in the nucleus, not only in Giemsa preparations but also in those stained after Heidenhain (Diagram X, Fig. 15, Diagram XI, Figs. 18 and 19). The undulating membrane is not broad and consequently not much curved. When the flagellate is

going to divide it becomes much broader (Diagram XI, Figs. 20—23) when the blepharoplast and nucleus divide (Fig. 22) a new flagellum is formed alongside the old one (Fig. 23) and at last the whole cell is divided (Fig. 24).

In the non-dividing forms two types, a slender and thick one, may be distinguished (Figs. 18—19), but this dimorphism has now been so often found that we do not think it has any special significance. In Fig. 14 (Diagram X) a very large type, which hardly deserves the name "Small trypanosome," is drawn; we do not understand its significance but merely mention it because it was sometimes encountered.

That the small trypanosomes are really directly connected with the small oval forms and Crithidiae is shown also in Fig. 16 (Diagram X) which represents a rosette showing dividing and small oval forms (*a*) intermediate forms between small oval forms and crithidiae, (*b*) a stout crithidia, (*c*) and two forms of which one is a slender crithidia, and the other an intermediate form between this one and the small trypanosome, (*d*) corresponding with Fig. 16 (Diagram XI).

These small trypanosomes are particularly interesting because they resemble the forms found by Chagas in the salivary glands of *Conorrhinus* infected with *Schizotrypanum cruzi*. There is however the great difference (apart from the place they occupy in the invertebrate host) that *T. lewisi* exhibits only one sort of development leading to the formation of the small trypanosomes, whereas in *Schizotrypanum* these forms are produced by a special development, different from that producing only crithidiae and round forms which are considered by Chagas to be merely cultural forms.

This was the last stage of development we found; neither in the Series F nor in fleas taken haphazard from the infected rat at different times could we find anything beyond this stage.

Comparison with cultural forms. Diagram XII shows some of the characteristic forms out of a culture of *T. lewisi* on Novy and MacNeal's medium. Figs. 1—3 are large and small crithidiae. Figs. 1 and 2 show very distinctly the mode of division of the flagellum; only the basal part is divided and so a short flagellum is formed which grows out. Figs. 4—8 show round forms in different stages of division. The blepharoplast first divides and seems to play the part of a centrosome during the subsequent division of the nucleus in the same way as described by França, but we think that, at least in this case, this function of blepharoplast is only an apparent one because sometimes

the nucleus is seen dividing without any direct relation to the blepharoplast.

The resemblance of these forms with *Crithidiae* and round forms from the flea's gut is indeed very striking and we do not hesitate to consider these stages of the development of *T. lewisi* in the flea as merely natural culture forms. This cannot be said however of the "small trypanosomes," they have never been observed in the culture either by us or by any other authors.

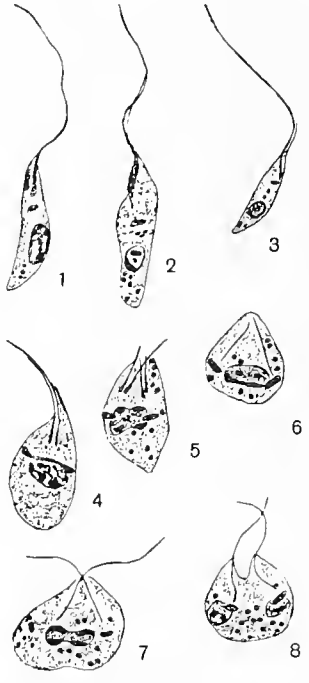

Diagram XII.

Figs. 1—3. Thick and slender crithidiae. *G.* Figs. 4—8. Dividing round forms. *G.*

Comparison with species of Crithidia described in different fleas.

Flagellates in fleas have been described by Swingle, Balfour and MacKinnon. Swingle considers the flagellates, found in the gut of fleas fed on rats infected with *T. lewisi*, as developmental stages of the latter flagellates. We gather from his description that he found small oval and round forms, but he did not trace out the whole cycle. Balfour found small, large oval, and round forms in *Pulex cleopatrae*, perhaps also small trypanosomes, but this point is not clear, because his preparations were obviously not well stained and so no flagella are to be seen.

Miss MacKinnon found in *Ctenophthalmus agyrtes* and *Hystrichopsylla talpae* two flagellates which in many stages of their development perfectly resemble our small oval and round forms.

We were able to find in *Ctenocephalus serraticeps* flagellated forms (Diagram XIII) which are almost identical to the small oval and round forms described here as developmental stages of *T. lewisi*; the same is the case with *Crithidia ctenophthalmi* of Patton and Strickland (1908).

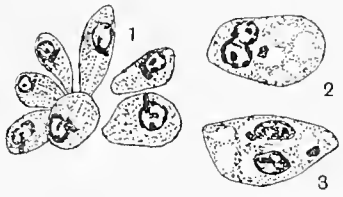

Diagram XIII.

Flagellates found in the hindgut of *Ctenophthalmus canis*. *G.*

Some of these fleas (*Ctenophthalmus agyrtes, C. serraticeps*) may attack rats and so it is possible that some of these *Crithidiae* or herpetomonads are really developmental stages of trypanosomes, but even if we consider all these flagellates to be natural forms we do not think that this invalidates in the least our view that the forms we found in the fleas fed on an infected rat, are really developmental forms of *T. lewisi*. The resemblance with the natural forms is very striking, but not greater than the resemblance with the cultural forms of *T. lewisi* and nobody will doubt that the latter are connected with *T. lewisi*. One point remains to be considered; two out of sixty of our control fleas were infected, the flagellates found in their gut belonging all to the type of the small oval forms, so one might consider these forms not to belong to the cycle of *T. lewisi*. This however is a mistake: the small oval forms were found after the seventh day in 15 fleas out of 83 = 18 %. In the control fleas they were found only in 3·3 %, so the only possibility is that a part of the small oval forms found in fleas of Series F did not belong to the cycle of *T. lewisi*.

Summary. Diagram XIV will serve better than words to give a summary of the life-cycle of *T. lewisi*, as observed by us in *Ceratophyllus fasciatus*. The following table (p. 379) gives the measurements of the different types described (out of the flea and of cultures).

As already stated, the small trypanosomes seem to be in the final stage of development, but we are unable to say if these are the

forms which are reinoculated into the rat when an infected flea is feeding on it, and so produce infection. We tried to find this or any other stages of development in the salivary glands or probosces of heavily infected fleas, but never succeeded in finding anything.

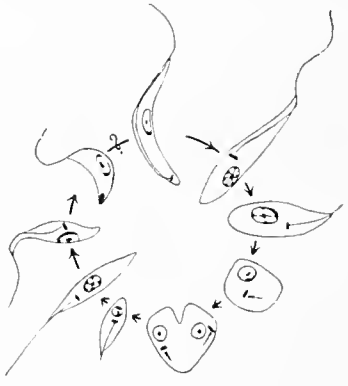

Diagram XIV.

Synopsis of the life-cycle of *T. lewisi* in the gut of *Ceratophyllus fasciatus.*

Apart from these considerations, the fact remains that *T. lewisi* undergoes a well-marked development with a definite conclusion in the gut of *Ceratophyllus fasciatus,* a development which cannot be merely considered as a natural culture, because apart from forms found also in cultures, stages of development were observed (small trypanosomes) which were never found in culture.

4. *Development of T. lewisi in the rat louse (Haematopinus spinulosus).*

The work on the development of *T. lewisi* in the invertebrate host has been done merely in connection with the rat louse; the papers referring to this subject have been quoted in the beginning of this article.

The development in the louse is a very irregular one and is not to be compared with that which takes place in the flea. As we have seen, the rate of infection in fleas fed only once on an infected rat is 36·9%. If fleas are taken haphazard from an infected rat almost every specimen shows developmental forms. This is not the case with the louse. During the summer of 1909, 270 lice were dissected in Cambridge, but only normal and degenerated forms were to be found in the gut. In the early spring of 1910, 50 lice were dissected in Amsterdam with

TABLE III.

Form	Alcohol fixation of wet films—Giemsa stain						Corrosive-alcohol fixation of wet films—Heidenhain stain					
	Length			Breadth			Length			Breadth		
	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum
Normal	17·5	—	—	1·0	—	—	12·5	—	—	1·0	—	—
Intermediate forms between normal tryp. & crithidiae	15·6	20·0	12·5	1·6	2·0	1·3	—	—	—	—	—	—
Slender crithidiac ...	13·4	16·3	10·5	1·0	—	—	10·0	—	—	1·0	—	—
Thick crithidiae	11·3	15·0	7·5	1·85	2·0	1·8	10·0	—	—	1·65	1·8	1·5
Large oval forms ...	9·03	12·5	7·3	2·32	2·8	2·0	8·65	9·0	8·3	2·4	2·5	2·3
Non-dividing round forms ...	5·1	8·3	4·0	3·7	5·3	2·5	4·4	5·1	4·0	3·47	4·0	3·3
Dividing round forms ...	6·02	7·5	4·8	4·7	7·0	2·8	4·5	4·8	4·3	5·25	6·0	4·5
Small oval forms ...	5·85	8·5	4·8	1·6	2·0	1·3	4·3	5·3	3·8	1·9	2·3	1·3
Intermediate forms between small oval f. & crithidiae	9·65	10·0	9·3	2·15	2·3	2·0	6·9	7·8	6·0	1·8	2·5	1·3
Crithidiae	12·5	14·5	11·5	1·9	2·5	1·83	10·5	—	—	1·5	—	—
Intermediate forms between crithidiae & small tryp.	8·23	10·0	7·3	1·47	2·0	1·0	6·7	6·8	6·5	1·17	1·5	1·0
Small tryp.	8·6	10·8	7·5	1·85	2·8	1·0	9·8	11·3	8·3	1·4	2·0	0·8
Dividing round forms of cultures	5·42	6·3	4·5	4·72	6·5	3·5						
Slender crithidiae of cultures	7·0	—	—	1·3	—	—						
Thick crithidiac	10·25	12·5	8·0	2·05	2·3	1·8						

N.B. The measurements are expressed in microns (μ). In the case of the flagellated forms the length of the free flagellum is *not* taken into account.

the same negative result; in both cases the rats were in the chronic stage of infection, *i.e.* the trypanosomes in their blood did not divide any more. In May 1910 we at last found lice showing developmental stages on the insects occurring on a rat in the last period of the infection. Fifty-two lice were dissected; 33% were not infected at all, 43% showed only normal and degenerating forms, 23% showed developmental forms. We are unable to state what the influences are which favoured the development. Generally these forms were only to be found in lice which had not sucked blood quite recently (gut with black contents). We often kept the lice for 24—48 hours in a moist chamber at 13—16° C. (after 48 hours all the lice had died) and were then generally able to find the developmental forms in the black contents of the hindgut. Manteufel (1909) was also able to find these forms under similar conditions. During the course of this experiment our rat lost its trypanosomes suddenly. Directly afterwards the normal forms disappeared, but the developmental forms were to be found till three days afterwards. This corresponds with the fact mentioned by Manteufel that the lice may be infective even five days after feeding. It remains to be determined if these developmental forms found in the louse are really connected with *T. lewisi* or if they are perhaps natural flagellates. The control observations are very difficult in this case; as has been pointed out already, developmental stages may not be found even in a large number of infected lice, so it is not enough to dissect a large number of lice and make sure that no flagellated forms are to be found, because if there is a natural infection it is very scanty. In our experiment the condition of the production of crithidiae being evidently very favourable, there was no reason (supposing that these forms were natural ones) why they should disappear after the rat ceased to be infective. We therefore continued dissecting lice for ten days after that time. Developmental forms were to be found during the first three days, but not later—for 61 lice were dissected with negative results. Although the number of our controls was not so large as that of Breinl and Hindle, we think we may safely conclude that the crithidiae are indeed connected with the trypanosomes, because their appearance and disappearance were so manifestly connected with presence or absence of trypanosomes in the rat on which the lice fed. (Table IV shows the rate of infection of each day.)

The course of infection being so very irregular, and it being impossible to keep the lice off the rat for more than 48 hours, we could not follow the changes occurring in the trypanosomes as regularly as in

TABLE IV. (*Series II—I.*)

Day	Infected lice: With development	Infected lice: Without development	Uninfected lice	
1	0	6	1	
2	2	5	1	
3	0	1	0	
4	3	2	0	
5	0	2	0	
6	1	2	0	
7	3	4	0	
10	1	0	2	*
11	2	0	13	
12	0	0	9	†
13	0	0	6	
14	0	0	6	
15	0	0	3	
16	0	0	3	
17	0	0	7	
18	0	0	6	
19	0	0	6	
20	0	0	6	
22	0	0	6	
23	0	0	3	

* The rat lost its trypanosomes between the 7th and 10th day.

† Beginning of the control series. Last day on which developmental forms were found.

the flea experiment. We have not indicated the dimensions of the different forms because they are the same as in the flea.

The first changes are the same as those observed in the gut of the flea (Diagram XV). The blepharoplast wanders in the direction of the nucleus (Fig. 2) and the flagellate becomes more or less club-shaped (Figs. 3 and 4), but sometimes it remains thin (Fig. 5).

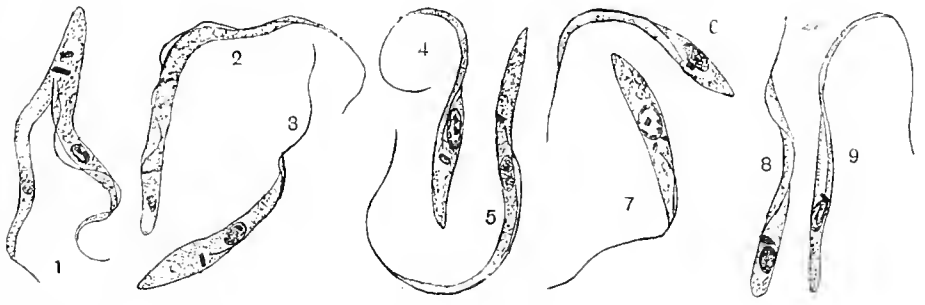

Diagram XV.

Fig. 1. Agglomeration of two normal forms. *G.*

Figs. 2—7. Nucleus and blepharoplast change their position. *G.*

Figs. 8—9. Thick and slender crithidiae. *G.*

The blepharoplast then passes the nucleus (which has been wandering to the posterior cell-end in the meantime) and so the crithidiae-type is produced. The crithidiae are thick or slender (Figs. 7 and 9) according to the flagellate having become club-shaped or not. During the first days, forms as drawn in Fig. 1 may be observed; they are due to agglomeration but might be taken for conjugation.

These changes are observable during the first two days. The forms now to be described in lice could not be found in a regular series, but using as a guide the knowledge we acquired in the course of the investigation of the flagellates in fleas, we have arranged our results accordingly.

All of these forms are found in the pyloric region of the hindgut. First of all the crithidiae and their flagella are shortened up (Diagram XVI, Fig. 1), the flagellates become broader (Figs. 4, 5) and at last rounded up (Figs. 6—8).

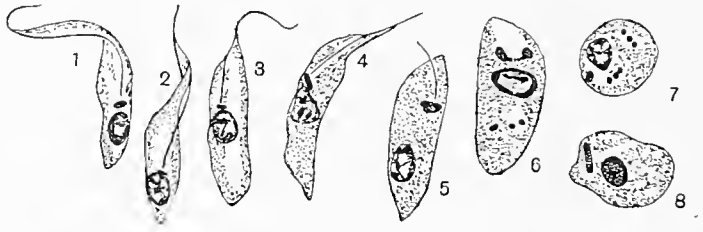

Diagram XVI.

Figs. 1—5. Transformation of crithidiae into large ovals. *G.*
Fig. 6. Large ovals becoming rounded up. *G.*
Figs. 7—8. Round forms. *G.*

The minute cytological structures of all these forms are the same as those described in the flagellates of the flea. A well-marked karyosome is generally, though not always, to be seen; the blepharoplast shows generally its chromatic and achromatic components quite distinctly. Fig. 6 shows the division of the latter, the two daughter blepharoplasts being united by an achromatic centrodesmose. This figure shows also that the two chromatic granules present in many blepharoplasts do not indicate the beginning of division because the two daughter blepharoplasts show already the double granule. The blepharoplast in Fig. 8 is also dividing. The flagella of the round forms do not stain well, generally they are not to be seen at all. The dividing nucleus of Fig. 6 seems to indicate that the division takes place in the same way as in the flea flagellates.

The short crithidiae with short flagellum are identical with the large oval forms of the flea's gut; they resemble Rodenwaldt's "ookinetes."

These large oval forms are generally united into rosettes (Diagram XVII, Fig. 1) with round forms, which confirms our view that the two are connected.

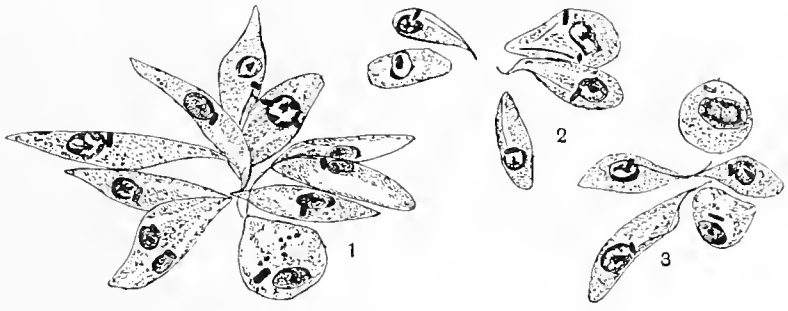

Diagram XVII. Different forms of rosettes. *G.*

Moreover, rosettes are found in which round forms and small crithidiae (identical with the small oval forms of the flea's gut) are present (Figs. 2 and 3), so we may conclude that these small oval forms are produced by the division of the round forms.

Apart from these forms, larger crithidiae with the nucleus and the blepharoplast in the front end of the cell (Diagram XVIII, Figs. 1, 2, 3) are frequently to be found; we do not know if they are to be ranged in

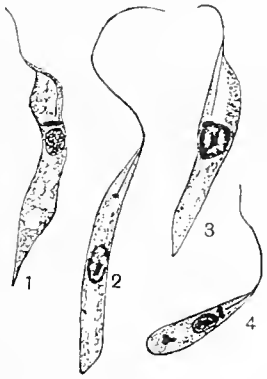

Diagram XVIII. Different forms of crithidiae and leptomonads. *G.*

the series before the large oval or after the small oval forms. Small crithidiae (or perhaps better herpetomonads) are also to be seen (Fig. 4) which may perhaps arise from the small oval forms.

The flagellates found in the louse taken from the rat three days after it lost its trypanosomes, show different changes which we think must be interpreted as degenerative. Large, dark-staining granules appear in the cells (Diagram XIX, Fig. 2), the nucleus stains very

palely, and at last nothing of it is to be seen but some irregular chromatic filaments, sometimes connected with extranuclear granules (Fig. 1); the blepharoplast also disappears.

We never were able to see anything beyond these stages, nor did the other authors who studied this subject. We utterly failed to find any form which could be identified with the "small trypanosome" forms in the flea.

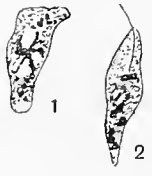

Diagram XIX. Degenerative stages.

When comparing these types with the cultural forms, we see that there is here a perfect resemblance, all the flagellated forms found in the gut of the louse may be found in the culture (cf. Diagrams XVI—XIX with Diagram XII). We think therefore that the development in the gut is a mere natural culture which lasts only for a short time, so long as the blood remains in the gut; but there is no real adaptation to the natural conditions of the gut. Perhaps the non-appearance of the small trypanosomes has something to do with this phenomenon.

During the first two days of the infection many degenerative forms are to be found, which are identical with those found in the flea's gut, so we abstain from describing them.

No signs of sexual processes were observed. The thick and slender crithidiae, described during the first two days of development, which might be considered as sexually differentiated forms, cannot be regarded as such owing to the fact that subsequent observation did not establish the occurrence of conjugation or any other sexual process.

We never could find any trace of development in the coelom as Baldrey and Prowazek did.

5. *Behaviour of T. lewisi in the gut of Ornithodoros moubata.*

After our positive results in trying to find developmental stages of *T. lewisi* in the gut of the flea and the louse, we tried to answer the question if there may be development in some other blood-sucking Arthropods. We chose a tick (*Ornithodoros moubata*) and the bed bug (*Acanthia lectularia*), because they suck large quantities of blood which

remains in the gut for a considerable time, thus affording a condition which seems to be particularly favourable for the production of a natural culture.

Six *Ornithodoros* were fed on a rat in the chronic stage of infection with *T. lewisi*, and were dissected during the following days. It is particularly difficult to make good stained preparations of the gut contents. The large amount of sphaero-crystals from the malpighian tubes seems very unfavourable for the production of a good staining.

Ornithodoros No. 1 (dissected after one day) and No. 2 (dissected after two days) showed only normal forms of trypanosomes, not worth mentioning. No. 3 (dissected after four days) contained trypanosomes showing different changes in the internal structure, which could only be interpreted as due to an incipient degeneration: hypertrophy of the blepharoplast (Diagram XX, Fig. 1), chromidia (Fig. 1), hypertrophy of the nucleus (Fig. 2), because the same phenomena are to be found in trypanosomes preserved in the refrigerator for 11 days.

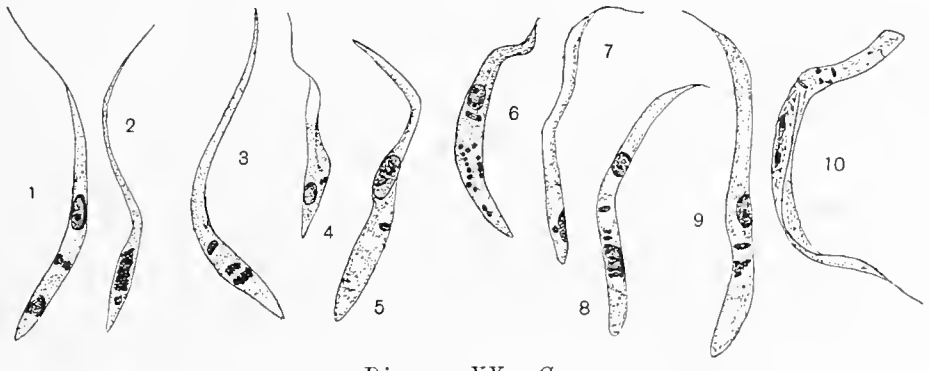

Diagram XX. G.

Figs. 1—2. Flagellate out of the gut of *Ornithodoros* No. 3.
Figs. 3—5. Flagellates out of the same tick, preserved for 24 hours in wet chamber.
Figs. 6—10. Flagellates out of the gut of *Ornithodoros* No. 4.

What remained of No. 3 was preserved in a moist chamber (at 13—16° C.) for 24 hours. After that time flagellates were still present in the gut, but the form was altered, club-shaped.

In stained preparations the hind end of the trypanosome was generally swollen up, the blepharoplast was situated in the neighbourhood of the nucleus (Fig. 5). Sometimes the blepharoplast even passed the nucleus (Fig. 4) so that a crithidia was produced. Degenerative signs, as fragmentation of the nucleus, were often to be observed (Fig. 3).

Ornithodoros No. 4 was dissected on the sixth day of the experiment. The flagellates found in the gut were very slender and showed hyperactive motion. In stained preparations most of them had two nuclei with the blepharoplast situated between them, exactly as in the forms we found in the culture between slide and cover-slip in the beginning of Series F (Diagram XX, Figs. 8—10).

Sometimes both nuclei are equally well developed (Fig. 8); sometimes the posterior nucleus seems to be degenerating, and in other cases both of them are very abnormal (Fig. 10). Beside these forms we found also long slender flagellates with the blepharoplast at the side of nucleus (Fig. 7), but no real crithidiae were to be found.

Fig. 6 shows a not infrequent type with the blepharoplast just behind the nucleus, the hind end being filled with dark-staining granules.

This tick No. 4 had been put in the incubator (22° C.) for two days in the hope of stimulating development, but it is possible that by this manipulation we only hastened the process of degeneration, for the gut of the same tick preserved in a moist chamber in the incubator for another night, did not contain any flagellates. Tick No. 5, put in the incubator for three days, and dissected seven days after feeding, did not contain any active flagellates. A like result was obtained with tick No. 6, dissected after eight days; this tick was put in the incubator for three days and then replaced at 13—16° C. for one day.

The trypanosomes are consequently kept alive in the gut for six days, in which time they undergo degenerative changes very much like those occurring in trypanosomes kept for some time in the refrigerator, *i.e.* the gut is not favourable to any development but checks the growth of bacteria, which should otherwise have destroyed the trypanosomes much sooner. It is an interesting fact that although there is no development in the tick, the longevity there is greater than in the louse, where development takes place[1].

6. *Development of T. lewisi in the gut of Acanthia lectularia (bed bug).*

Our observations in this connection are incomplete, nevertheless they appear worth recording. As controls thirteen bugs were dissected to

[1] There being no development at all, controls were not necessary; the crithidiae found in tick No. 3 cannot be considered as developmental.

see if the gut contained any natural flagellates, but nothing was to be seen. Professor Nuttall informs us that he has dissected at least 80 bugs for other purposes but never found any trace of natural flagellates. So we may be sure that the forms found in our bugs really belonged to *T. lewisi*.

Two bugs were fed on a lewisi-rat in the chronic stage of infection.

On the third day after the infective meal, bug No. 1 was dissected. The posterior end (black blood) and the anterior end (red blood) of the midgut were swarming with flagellates.

Stained preparations showed:

(*a*) Forms with a broad hind end, the blepharoplast situated close to the nucleus (Diagram XXI, Figs. 1—2).

(*b*) Forms with a hind end much broader than in type *a* (Figs. 3—5), the blepharoplast situated behind, beside or before the nucleus. The front end of the flagellate is generally very slender. Fig. 4 represents a rather extreme form, which was to be seen also in the living preparations.

(*c*) Ordinary crithidiae (Figs. 6—8).

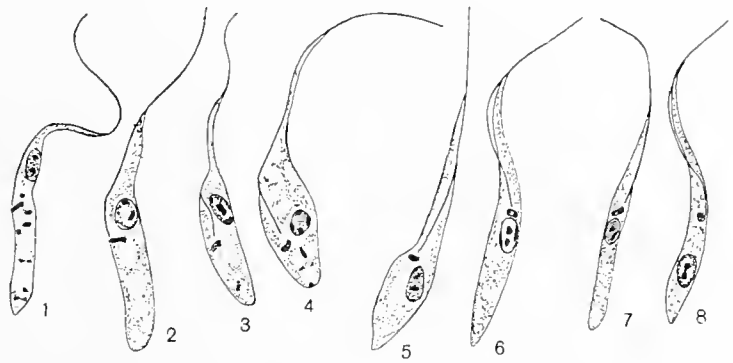

Diagram XXI. G.

Figs. 1—5. Formation of club-shaped forms.
Figs. 6—8. Crithidiae.

Bug No. 2 was dissected nine days after feeding (kept in a gauze bag at 13—16° C.) and contained flagellates only in the anterior part of the midgut. They were of the true crithidiae type, possessing a slender rod-like body, no apparent undulating membrane and a long free flagellum. They were not so frequent as in bug No. 1. The stained preparation was unhappily destroyed, the blood film being washed off, but judging from the living specimens the aspect was the same as that of the flagellates represented in Figs. 6—8. No club-shaped forms

were to be seen. In the stained preparations of the posterior part of the midgut no trace of crithidiae or round forms could be found. There may or may not be some further development in bugs kept for a longer time, but our experiments show at least that there is some development and a survival for at least nine days.

Summary.

From the results of our experiments we may conclude that the development of *T. lewisi* outside the invertebrate host is not confined to one species or genus, but may take place at least in the rat louse and the rat flea and also (though perhaps more incompletely) in the bed-bug. We see further that development needs not always to be combined with longevity in the invertebrate host, but that sometimes life without development may be longer than with it (behaviour in the tick compared to that in the louse).

When we compare the behaviour of *T. lewisi* in the four invertebrate hosts studied here, we see that the most complete cycle takes place in the flea, where forms are produced which are never found in cultures. In the louse the development may be truly described as a natural culture; in the bug, the development (as far as we could judge by our incomplete experiment) does not even produce all the cultural forms (only the crithidiae) and at last in the tick the trypanosomes do not develop at all but are only preserved for some time.

We finish this paper by thanking Professor Nuttall for the liberal way in which he enabled us to carry out these experiments, and for the keen interest he always took in our work.

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Chagas (1909). Über eine neue Trypanosomiasis des Menschen. Studien über Morphologie und Entwickelungscyclus des *Schizotrypanum cruzi* n. gen. n. sp. Erreger einer neuen Krankheit des Menschen. *Memorias do Instituto Oswaldo Cruz*, vol. i.

MacKinnon (1909). Note on two new flagellate parasites in fleas. *Parasitology*, vol. II.

Manteufel (1909). Studien über die Trypanosomiasis der Ratten, etc. *Arb. a. d. Kaiserl. Gesundheitsamte*, XXXIII. Heft 1.

Minchin and Thomson (1910). The transmission of *Trypanosoma lewisi* by the rat flea (*Ceratophyllus fasciatus*). *Proc. Roy. Soc. London*, B. vol. LXXXII.

Patton and Strickland (1908). A critical review of the relation of blood-sucking invertebrates to the life-cycles of the Trypanosomes of Vertebrates. *Parasitology*, vol. I.

Prowazek (1905). Studien über Säugetiertrypanosomen. *Arbeit. a. d. Kaiserl. Gesundheitsamte*, vol. XXII.

Rodenwaldt (1909). *Trypanosoma lewisi* in *Haematopinus spinulosus*. *Centralbl. f. Bakteriol.* Abt. I, Orig. vol. LII.

Swellengrebel (1910). Fixing and staining of *Trypanosoma lewisi*. *Parasitology*, vol. III.

Swingle (1907). Some studies on *Trypanosoma lewisi*. *Trans. Amer. Microsc. Soc.* vol. XXVII.

PUBLICATIONS RECEIVED.

BOOKS.

BRAUN, M. and LÜHE, M. (1910). *A Handbook of Practical Parasitology*, translated by Linda Forster. 208 pp. 100 text figs. 26 × 16 cm. Cloth. London: John Bale, Sons & Danielsson, Ltd., 83 Great Titchfield St. W.

The book is divided into three parts dealing respectively with Protozoa (pp. 1—91), Helminthes (pp. 92—173) and Arthropoda (pp. 174—192) and is provided with a good index. The value of the book for laboratory workers consists in the large amount of information it contains regarding technical methods.

CASTELLANI, A. and CHALMERS, A. J. (1910). *Manual of Tropical Medicine*, 1242 pp. with 14 coloured plates and 373 text figures. 22 × 15 cm. Cloth. London: Baillière, Tindall & Cox, 8 Henrietta St., Covent Garden.

The first thing that strikes the reader in opening this book is the wealth of new and excellent illustration, a great deal of which is original, the rest obtained from many sources to which the authors give due credit. The book contains a great deal that is not to be found in other works on tropical medicine and it should consequently find a wide circle of readers. The various chapters are supplied with many references to the literature which has been brought up to date in a remarkable manner considering the dimensions of the book and especially the fact that the authors reside in Ceylon. Both the authors and publishers are to be congratulated upon the excellent appearance of the book; it will certainly be a great help to students of tropical medicine.

DANIELS, C. W. (1910). *Tropical Medicine and Hygiene*, with a chapter on Snakes by A. Alcock, F.R.S. Part II. Diseases due to the Metazoa. 283 pp. with 1 pl. and 98 text figures. 22 × 15 cm. Cloth 7*s.* 6*d.* London: John Bale, Sons & Danielsson, Ltd., 83 Great Titchfield St., W.

This book, which is destined to appear in three parts, provides a concise description of parasitic diseases, their mode of spread and their prevention. Part II is divided into sixteen chapters of which the first thirteen deal with Vermes. Chapter XIV gives a résumé of prophylactic measures directed against diseases due to worms. Chapter XV deals with Leeches and Arthropoda—little more than a page is devoted to Leeches whilst no reference is made to fleas or ticks which will doubtless receive attention in another part of the book.

An account of snakes and snake-venoms by Colonel Alcock is contained in the last chapter and a very full index concludes the volume. We have no doubt that the work will receive a warm welcome from students of tropical medicine. Dr Daniels and Col. Alcock who have for years been associated with the London School of Tropical Medicine will know the needs of those taking up the study of the subjects with which this book deals.

MARTIN, LEBOEUF and ROUBEAUD (1909, received IV. 1910). *Rapport de la Mission d'Études de la Maladie du Sommeil au Congo Français* 1906—1908 (Société de Géographie). pp. vii. + 721, 8 plates (6 coloured), 1 portrait and 136 text figures, 1 large coloured map. 28 × 20 cm. Cloth. Paris: Masson et Cie.

The investigations of the French mission for the study of sleeping sickness in the French-Congo were rendered possible through the enterprising chairman of the Geographical Society, M. le Myre de Vilers, whose portrait serves as a fitting frontispiece to the very handsome volume before us. The volume opens with an explanatory note by Roux and a short preface commencing with the statement that "The future of the Congo, economically considered, is bound up with the question of human trypanosomiasis."

The volume is divided into eleven chapters as follows: I. Organization and programme of the Mission.—II. Geographical Distribution of sleeping sickness and of biting flies in the Congo (pp. 27—235).—III. The manner in which the disease is spread. Epidemics of sleeping sickness. Occurrence in families and sporadically.—IV. Microscopic diagnosis of human trypanosomiasis.—V. Enlarged lymphatic glands in sleeping sickness.—VI. Clinical study of human trypanosomiasis.—VII. Therapeutics.—VIII. Researches upon the structure and biology of *Glossina palpalis*: habitat, migration, food, reproduction, the larva, pupa etc., means of extermination.—IX. Pathogenic Trypanosomes and *Glossina palpalis* (Historical—study of Trypanosomes in natural infection—study of experimental infection in Glossinae—relation of mammalian pathogenic Trypanosomes to the Leptomonads of insects' intestines—the etiological part played by *Glossina palpalis*. Experimental study—bibliography).—X. Prophylaxis.—XI. Animal trypanosomiases (trypanosomiases of animals in the French Congo and of mammals in Sangha-Logone-Ouhame)—on relapses following treatment in human trypanosomiasis.

The numerous illustrations relate to the Laboratory at Brazzaville, experimental methods, human cases, habitats of Glossina, the anatomy of Glossina, various Trypanosomes, etc. In short the Report is one of great importance to all concerned in the study of sleeping sickness and allied diseases.

SURCOUF, J. M. R. (1909, received IV. 1910). *Étude Monographique des Tabanides d'Afrique* (*Groupe des Tabanus*). Avec le Concours de Miss G. Ricardo. 260 pp., 3 coloured plates, 26 text figures and 22 maps. 28 × 19 cm. Paris: Masson et Cie., 120 Boulevard St.-Germain (VI e).

This monograph will be most welcome to all who are concerned in the study of the blood-sucking insects occurring in Africa and which may be regarded as potential carriers of disease. The author, M. Surcouf, is "chef des travaux de Zoologie au Laboratoire colonial du Muséum de Paris" and he has been aided in his work by Miss G. Ricardo of the British Museum, one of our

leading authorities on Tabanids in this country. The excellent form in which the publication appears is due to financial aid obtained from the Institut Pasteur.

We can but congratulate the authors upon this very useful and well-illustrated work, in which they describe no less than 115 species of *Tabanus* in a masterly manner, giving keys for their determination, notes upon their biology, and maps relating to their geographical distribution, a full bibliography being appended.

PERIODICALS.

Bulletin of Entomological Research. Vol. I. Part I. pp. 1—88 (April, 1910). Part II. pp. 89—190 (July, 1910). Issued by the Entomological Research Committee (Tropical Africa) appointed by the Colonial Office. Editor: The Scientific Secretary (Guy A. K. Marshall). London: Longmans, Green, & Co., 39 Paternoster Row, E.C. and Taylor & Francis, Red Lion Court, Fleet St., E.C. Price per part, 4*s.*

Following upon a Meeting held at the Colonial Office in March 1909 the Colonial Office appointed a body to be known as the Entomological Research Committee for Tropical Africa:—*Chairman:* The Earl of Cromer, G.C.B., G.C.M.G., O.M. *Members:* Lieut.-Col. A. Alcock, C.I.E., F.R.S.; Mr E. E. Austen; Dr A. G. Bagshawe; Dr J. Rose Bradford, F.R.S.; Col. Sir David Bruce, C.B., F.R.S.; Dr S. F. Harmer, F.R.S.; Dr R. Stewart Macdougall; Sir John Macfadyean; Sir Patrick Manson, K.C.M.G., F.R.S.; Mr R. Newstead; Prof. G. H. F. Nuttall, F.R.S.; Prof. F. B. Poulton, F.R.S.; Lieut.-Col. D. Prain, C.I.E., F.R.S.; Mr H. J. Read, C.M.G.; The Hon. N. C. Rothschild; Dr D. Sharp, F.R.S.; Dr A. E. Shipley, F.R.S.; Mr S. Stockman; Mr F. V. Theobald; Mr C. Warburton. *Scientific Secretary:* Mr Guy A. K. Marshall. *Secretary:* Mr A. C. C. Parkinson.

The Committee was formed with the object of furthering research in entomology in British tropical Africa. The sphere of usefulness may however with time be extended so as to cover a larger field within the Empire. Collections of all kinds of insects and ticks will be formed, special attention being given in the first instance to disease-transmitting forms affecting man and domesticated animals and to insects causing injury to cultivated plants. Efforts are to be made, at the instance of the Committee, to further our knowledge regarding the life histories and geographical distribution of these pests, and it is hoped that resident officials in the tropics will become interested in furthering the work especially since its great practical importance has received recognition from Government. Since the Committee was formed no time has been lost. Two entomologists have been sent to East and West Africa respectively and through their personal efforts and the stimulus they have brought to residents in the regions they have been and are traversing a vast and valuable material is being collected which will be distributed to experts for purposes of study and to suitable institutions.

The Bulletin to which this review relates is issued with the object of publishing the results of the work done by those associated with the Committee. Owing to the financial aid given by the Colonial Office, the Bulletin—which in appearance somewhat resembles *Parasitology*—will be issued at what is practically cost price, the object being to make it accessible to all who may be interested in the subjects with which it deals.

The two first numbers contain the following papers:

No. 1. On the larval and pupal stages of West African Culicidae by W. Wesché (pp. 1—50, 8 plates).—The Study of Mosquito Larvae by W. M. Graham (pp. 51—54).—Notes on the blood-sucking Diptera met with in E. and S.E. Abyssinia by R. E. Drake-Brockmann (pp. 55—57).—Notes on two W. African Hemiptera injurious to Cocoa by G. C. Dudgeon (pp. 59—61, coloured plate).—On scale Insects (Coccidae) etc. from the Uganda Protectorate by Robert Newstead (pp. 63—69, 2 text figures).—A new genus and two new species of African Fruit-flies by E. E. Austen (pp. 71—77, 2 text figures).—A new species of *Cordylobia* etc....the larvae of which are subcutaneous parasites in man and other mammals by E. E. Austen (pp. 79—81, 1 text figure).—On the parasites of two species of W. African wild silk-worms by G. C. Dudgeon (pp. 83—84, 1 fig.).—Note on a Method of Destroying Tsetse-flies.—Lists of collections received.

No. 2. A Synopsis of the fleas found on *Mus norwegicus decumanus*, *Mus rattus alexandrinus* and *Mus musculus* by the Hon. N. C. Rothschild (pp. 89—98, 28 figs.).—Some observations on the bionomics of *Tabanus par*, Walker, and *Tabanus taeniola*, Pal. de Beauv. by H. H. King (pp. 99—104, coloured plates). A short survey of the more important families of Acari by A. C. Oudemans (pp. 105—119, 22 figs.).—A Mealy Bug injurious to the Libbek Trees of Cairo by F. C. Willcocks (pp. 121—137, 9 figs. 1 map).—Short notes on various related matters and Lists of collections received.

Malaria e Malattie Affini. Organo ufficiale della Lega Nazionale contro la Malaria. Direttori Onorari: Guido Bacelli, Camillo Golgi, G. Battista Grassi. Direttore effetivo Prof. U. Gabbi, Dr G. Tropeano, Dr G. Montoro. Anno. I Nos. 1—2, June 1910, pp. 1—64.

Rome [Editorial and Publishing Office]: 62 Via Farini.

Price: 8 Liras, abroad, published twice a month.

In April 1910, the Italian National League against Malaria held a meeting in Rome which was attended by representatives of the provincial committees. It was decided at the meeting to publish a journal dealing with malaria and allied diseases and which would serve as a centre for the publication of the work done by the various committees and by the league.

The contents of the first number are as follows: "Il Bollettino della Lega" by the Directors.—"Ciò che si deve tenere sopratutto presente nella lotta contro la malaria" by B. Grassi.—"Contributo sperimentale allo studio dell' azione della chinina sui reni degli animali sani" by E. Brugnatelli.—"Le infezioni simulatrici della malaria" by U. Gabbi.—Accademie e Congressi.—Sunti e riviste.—Vita professionale.—Notiziario.—Concorsi e condotte.

Memorias do Instituto Oswaldo Cruz, I. fasc. II. 1909, Rio de Janeiro. The new Memoirs are published in Portuguese and German printed in columns side by

side. The number contains the following papers which will necessarily interest parasitologists particularly :

Zur Entwicklung von *Spirochaeta gallinarum* by S. v. Prowazek.—Ueber die Vermehrung der Bacterien in den Culturen. I. Die Constante ihrer Geschwindigkeit by A. Godoy.—*Echinostomum crotophagae* n. sp. a new parasite of the blue Anú ; *Crotophaga major* (1 pl.) by G. de Faria.—Formdimorphismus bei Ciliaten Infusorien (1 pl.) by S. v. Prowazek.—Ueber den Nachweis von Antigen und Antikörper durch Complementablenkung by A. Moses.—Beitrag zur Kenntniss der brasilianischen Simuliumarten by A. Lutz.—Variola-Untersuchungen (2 pl., 2 figs.) by S. v. Prowazek and H. de Beaurepaire Aragão.—Also the paper by C. Chagas (see below).

REPRINTS.

Chagas, C. (1909). *Nova Tripanozomiaze Humana* Estudos sobre a morfolojia e o ciclo evolutivo do *Schizotrypanum cruzi* n. gen., n. sp., ajente etiolojico de nova entidade morbida do homem. *Memorias do Instituto Oswaldo Cruz*, I. Reprint 62 pp., 4 coloured plates (Rio de Janeiro).

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